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ETIOLOGY OF OSTEOCHONDROSIS OF SWINE

by

JOHN JOSEPH BRENNAN

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

~~OF DOCTOR~~ OF PHILOSOPHY

IN

ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL 1986

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ETIOLOGY OF OSTEOCHONDROSIS OF SWINE submitted by JOHN JOSEPH BRENNAN in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ANIMAL NUTRITION.

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ABSTRACT

Ninety-six crossbred gilts and 96 crossbred boars averaging 20 kg were assigned to a factorial arrangement of three dietary calcium-phosphorus (Ca-P) levels and two slaughter weights (100 or 130 kg). The three respective dietary Ca-P levels were 0.60-0.50, 0.81-0.63 and 1.05-0.82 % during the growing period and 0.50-0.40, 0.72-0.54 and 0.94-0.70 % during the finisher period. There was no significant effect of dietary Ca-P regimen on growth performance of pigs during the 20 to 100 kg period or on the incidence and severity of osteochondrotic lesions. Metacarpal bending moment and femoral ash content increased ($P<0.05$) in response to elevated dietary Ca-P levels. Boars had more severe ($P<0.01$) osteochondrotic lesions of the elbow joint and a lower ($P<0.001$) femoral ash content than had gilts. Correlation coefficients between cartilage soundness scores and bone ash content or bending moment were non-significant ($P>0.05$). The results suggest that while bone ash content and bending moment respond positively to elevated Ca-P levels in excess of NRC (1979) recommendations, the incidence and severity of joint lesions and leg soundness scores are not reduced by maximising bone mineralization.

The left half-carcasses from each of 96 boars and 96 gilts which were slaughtered at 100 or 130 kg bodyweight were used to study the relationship between carcass traits and the severity of osteochondrosis. Nine muscles from the proximal pelvic group, the femur and the tibia were each dissected and weighed. Knee joint soundness scores for the 130 kg animals were correlated ($r=0.28$; $P<0.01$) with mass of the adductor femoris relative to total proximal pelvic muscle mass. Muscle to bone ratio (based on partial dissection) and carcass length were not significantly ($P>0.05$) correlated with cartilage damage at any bone site examined. Therefore, carcass characteristics and lean tissue growth rate were poor predictors of OC in swine, but knee joint damage was associated with some localised changes in muscle distribution.

Sixty-seven pigs were weaned at five weeks of age and assigned to pens with either plastic coated expanded metal or woven wire flooring. Floor type did not have any significant ($P>0.05$) effect on pig performance. Foot lesions were most severe ($P<0.01$) in starter pigs

which were penned on woven wire flooring. There was also a tendency ($P<0.06$) towards more severe foot lesions in 95-kg gilts which were penned on woven wire flooring. The outer digits of the feet of these gilts had more severe lesions ($P<0.01$) than had the inner digits. The severity of osteochondrosis was not significantly ($P>0.05$) affected by floor type.

Twenty crossbred gilts (20.5 kg) were assigned to a mechanical stress or control treatment. Mechanical stress was achieved by placing a weight of 15% of bodyweight on the loaded animals and this ratio was maintained by weekly increases in load weight. Lesions associated with a reduced proteoglycan staining occurred at the articular surface of the medial humeral condyle of 50% of the loaded animals but were not observed in the controls. Performance, percent of time when pigs were standing, and elbow joint synovial fluid hyaluronate concentrations and cathepsin B-like activity were not significantly affected by loading.

Eighteen lame boars were equally assigned to treatment with saline or glycosaminoglycanpolysulfate (GAGPS) based on initial weight and leg soundness score. Leg soundness score was improved by GAGPS therapy but performance was unaffected. The hyaluronic acid concentration in the synovial fluid of the elbow joint increased ($P<0.06$) in response to GAGPS. Using Sepharose 2B gel chromatography, an increased percentage of aggregated proteoglycans was observed in fraction A1 proteoglycans from the joint cartilage of the GAGPS-treated boars vs the control boars. Joint soundness scores were not significantly affected by treatment.

ACKNOWLEDGEMENTS

The experiments described herein were supported by the Alberta Pork Producers Marketing Board, the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Alberta Agricultural Research Council.

I wish to thank Mr. Ed Maycher, Mr Bob Mascarin and Mr. Neils Neilsen for their excellent care and management of the animals used in my studies. The assistance of Ms. Inez Gordon and Mrs. Helen Murphy during carcass dissection is much appreciated. I am very grateful to Dr. G. Johnston and Dr. A. Perry of the Animal Health Division of Alberta Agriculture for their assistance with histological studies and to Dr. Bob Hardin and Mr. Ray Weingardt for their valuable help with statistical analyses.

I am indebted to my dear friend, Dr. T. Nakano, for sharing his expertise on osteochondrosis with me. Sincere thanks also to Dr. Frank Aherne for his constant guidance in my research and teaching endeavours and for his concern, enthusiasm and inspiration throughout my four years of study.

The encouragement and support given to me by my wife, Erika Weltzien, and my parents are much appreciated.

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I. INTRODUCTION

Leg weakness is a general term used to describe locomotory problems in swine. Reports from several countries indicate that up to 20% of boars in performance test stations are culled because of this condition (Harbison 1976). Many tissues contribute to the structural soundness of the pig but non-infectious degenerative joint disease is an important cause of lameness (Grondalen 1974a; Van der Valk et al. 1981).

Joint and growth plate cartilage consist of chondrocytes which are embedded in an abundant extracellular matrix of proteoglycans and collagen. Long bones grow by proliferation and maturation of chondrocytes followed by calcification of cartilage matrix in the joint cartilage and physis. Osteochondrosis is manifested as a partial failure of endochondral ossification in which vascular penetration does not occur. As bone growth progresses, cartilage is retained in subchondral bone and the resultant lesion enlarges. Osteoarthrosis is a condition in which normal ossification may occur but the articular cartilage surface is irregular or fractured. The articular cartilages most commonly affected are those of the distal humerus and distal femur which form the elbow and knee joints respectively (Grondalen 1974a). The distal ulnar growth plate also has a very high incidence of osteochondrosis.

Because osteochondrotic lesions are usually observed in the area of the chondro-osseous junction, it is not clear if the lesion originates primarily in cartilage or in subchondral bone. A number of cartilagenous defects can cause a failure of vascular penetration and Olsson (1978) has suggested that ostechondrosis results from abnormal growth and maturation of chondrocytes (i.e. dyschondroplasia). Nakano et al. (1986) suggested that lesions observed in the deep regions of femoral condylar cartilage would likely persist in the joint cartilage of the growing pig and result in a failure of endochondral ossification. Lesions of the distal ulnar epiphysis, closely resembling those occurring in ostechondrosis were induced by mechanical loading of the fore-legs of growing swine (Grondalen and Grondalen 1974) and were thought to result primarily from an interruption of metaphyseal blood flow. It is therefore possible that both cartilagenous and osseous lesions are primary causes of ostechondrosis although their relative importance is uncertain. Joint

lesions have been observed macrosopically in pigs as young as 4 weeks of age (Perrin et al. 1978) and histologically in newborn pigs (Hill et al. 1985). It is well established that the incidence and severity of ostechondrosis increases with age and virtually all pigs of 6 months of age have lesions at some bone site (Grondalen 1974a; Nakano et al. 1979a). The most severe forms of joint degeneration are observed in mature boars and sows (Reiland 1978a). The weight-bearing aspects of the medial condyles of the femur and humerus are predilection sites for the development of ostechondrosis in growing-finishing pigs but at birth the distribution of osteochondrotic lesions seems to be random for the sites thus far examined (Hill et al. 1985). This implies that although the condition is congenital, factors influencing endochondral ossification between birth and 6 months of age cause an accellerated development of ostechondrosis in the elbow and knee joints. Because ostechondrosis is manifested as a disturbance of bone growth its severity increases as bone growth progresses. It has been hypothesised that modern intensive methods of production and confinement housing of swine have increased the incidence of osteochondrosis (Grondalen 1977). Lack of exercise, rapid growth rate, conformational changes and nutritional factors have been cited as possible contributing factors in the etiology of this condition. Rapid weight gain in young pigs may increase mechanical stress on the weight-bearing regions of immature articular cartilage. Therefore, it was suggested that restricted feeding of pigs might help ameliorate leg weakness (Grondalen 1976). Reiland (1978b) observed a very low incidence of osteochondrosis in swine fed less than 60% of ad libitum feed intake. Milder restrictions of feed intake did not significantly reduce the incidence of osteochondrosis in 90 kg boars (Nakano et al. 1979b) or in boars, gilts and castrates slaughtered between 55 and 118 kg (Fell et al. 1970). Other studies (Perrin et al. 1978; Nakano et al. 1984) have shown that normal variations in growth rate of swine populations fed commercial diets are not significantly correlated with the severity of joint lesions. The low incidence of distal ulnar osteochondrosis in wild pigs (Fell et al. 1967) and the absence of the disease in Yucatan pigs (Farnum et al. 1984) suggests that genetically slow-growing pigs are less susceptible to this condition. Grondalen (1977) suggested that factors such as weakness of muscle and ligaments, abnormal exterior

conformation and joint shape induce local overloading in the joint and precipitate cartilage lesions. One recent study has indicated that the severity of osteochondrosis could be predicted from carcass characteristics (van der Wal et al. 1980).

Vitamins A and D are essential for normal bone growth. However, increasing dietary levels of vitamins A, D and E above the usual recommended levels for swine did not prevent development of joint lesions (Walker et al. 1966; Grondalen 1974c; Reiland 1978c). Vitamin C is essential for biosynthesis of collagen which contributes to the tensile strength of cartilage. Walker et al. (1966) reported that supplementation of swine diets with vitamin C could not prevent the development of osteochondrosis. Pigs with joint damage had normal plasma ascorbic acid concentrations and normal urinary excretion of ascorbic acid. Nakano et al. (1983) reported that dietary supplementation of 350 or 750 ppm vitamin C did not significantly reduce the incidence of elbow or knee joint lesions in swine. Also, collagen content of knee joint articular cartilage was not increased by supplemental vitamin C. Dietary manganese and copper concentrations in excess of the requirement for optimium growth of swine did not reduce the incidence of lesions of the distal ulnar epiphysis in swine (Walker et al. 1966).

Because of the importance of calcium (Ca) and phosphorus (P) in bone structure and the skeletal abnormalities long associated with deficiency of these minerals it is often suggested that current National Research Council (NRC) (1979) recommended dietary levels of Ca and P for growing and finishing swine may not be adequate for "normal skeletal development" (Nimmo et al. 1980). In addition it has been suggested that improvement in growth rate and feed conversion efficiency of fast growing pigs may lead to inadequate daily intakes of Ca and P. Many studies have examined the effect of dietary Ca and P on bending moment and ash content of bone but a relationship between elevated bone breaking strength and joint soundness has not been established. There is very little information available concerning the effect of elevated dietary Ca-P levels on the severity of osteochondrotic lesions in swine.

The proteoglycan aggregates of articular cartilage are comprised of a hyaluronic acid backbone to which proteoglycan monomers are attached via a link protein. Each monomer consists of a core protein to which keratin sulfate and chondrotin sulfate chains are covalently bound. The abundance of sulfate groups causes a repulsion of adjacent glycosaminoglycan chains in the molecule and the binding of a large volume of water. Therefore the proteoglycan aggregates remain expanded and give the tissue its resilience and its ability to support weight (Maroudas 1979). A loss of proteoglycan staining has been observed histologically in osteochondrotic articular cartilage (Nakano et al. 1979b). In addition, a reduction in proteoglycan aggregate size occurs in the joint cartilage of adult pigs suffering from osteochondrosis (Nakano et al. 1985) or lameness (Simunek and Muir 1972) due to a failure of interaction of proteoglycan monomers with hyaluronic acid. The mechanisms responsible for these changes in proteoglycan structure are of vital importance to our understanding of the etiology of osteochondrosis. Because the synovial fluid is responsible for efficient lubrication of the articular cartilage and soft tissues of the joint, its composition could also have a significant effect on joint integrity and structural soundness of swine. This aspect of joint integrity has, however, received little attention in most studies of osteochondrosis.

The objectives of the studies described herein were to examine the effects of nutritional factors, growth rate, carcass characteristics, foot lesions and mechanical stress on the severity of osteochondrosis and on the biochemical composition of articular cartilage, synovial fluid and bone. The efficacy of treating lame animals was also investigated using articular cartilage proteoglycan structure, synovial fluid hyaluronic acid concentration and joint and leg soundness as response criteria.

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II. EFFECTS OF DIETARY CALCIUM AND PHOSPHORUS LEVELS ON PERFORMANCE CRITERIA, BONE BENDING MOMENT AND THE SEVERITY OF OSTEOCHONDROSIS AND LAMENESS IN BOARS AND GILTS SLAUGHTERED AT 100 OR 130 KG BODYWEIGHT [1]

A. INTRODUCTION

Osteochondrosis (OC) is a failure of endochondral ossification which causes bone and cartilage lesions in a majority of slaughter weight hogs (Grondalen 1974b). The elbow and knee joints are most commonly affected (Nakano et al. 1979a) and when lesions are severe, lameness occurs and affected breeding or potential breeding animals are usually culled. It has been suggested that maximium mineralization of bone during growth and development may be necessary if swine are to remain structurally sound (Kornegay and Thomas 1981). Van Kempen et al. (1976) suggested that maximisation of bone strength was preferable to maximisation of bone mineral content in swine. Many studies (Nimmo et al. 1980; Kornegay et al. 1981; Hickman et al. 1983; Maxson and Mahan 1983) have demonstrated the effects of dietary Ca and P levels on ash content and various mechanical properties of bone in swine, but there is little information available concerning the relationships between these bone parameters and the incidence of bone and joint lesions. One report (Nakano et al. 1981b) indicated that a generalised reduction in bone mineralization does not occur in osteochondrotic bones, and a study by Grondalen (1974a) suggested that daily intake of Ca and P in excess of the requirement for maximal average daily gain had no significant effect on the incidence of joint lesions in swine.

The Agricultural Research Council (ARC) (1981) recommended dietary Ca-P levels for grower and finisher swine which are almost 50% higher than current National Research Council (NRC) (1979) recommendations. It is possible therefore that we in North America are feeding inadequate levels of Ca and P. The situation may be further aggravated by the fact that many swine producers restrict the feed intake of animals kept for breeding without

[1]A version of this chapter has been accepted for publication. Brennan, J.J. and Aherne, F.X. 1986. Can. J. Anim. Sci.

adjusting the Ca and P levels of the diet.

The objective of this experiment was to determine the effect of dietary Ca and P levels on performance criteria, bone bending moment and cartilage and leg soundness of boars and gilts slaughtered at 100 or 130 kg body weight and restricted or ad libitum fed from 100 to 130 kg body weight.

B. MATERIALS AND METHODS

Ninety-six crossbred (Yorkshire x Landrace) gilts and 96 crossbred boars averaging 20 kg bodyweight were assigned to a factorial arrangement of three dietary Ca-P levels and two slaughter weights (100 or 130 kg). One Ca-P regimen was similar to that suggested by NRC (1979) for growing and finishing swine and was designated "low" (Table II.1). The second Ca-P regimen was similar to that recommended by ARC (1981) and was designated "moderate". The third treatment group was fed Ca-P levels calculated to be 30% greater than those recommended by ARC (1981) for growing and finishing swine and was designated "high". All pigs were fed the grower diets ad libitum from 20 to 55 kg and the finisher diets to 100 kg or 130 kg liveweight. At 100 kg liveweight, 16 boars and 16 gilts from each of the Ca-P treatment groups were selected at random and slaughtered. Fifty per cent of the pigs from each Ca-P treatment group that were slaughtered at 100 kg were given intramuscular injections (15 mg/kg bodyweight) of tetracycline hydrochloride at 35, 55 and 75 kg liveweight. The tetracycline was used as a bone marker and results will be presented in chapter III. Animals within each treatment group that were not slaughtered at 100 kg were either allowed feed ad libitum or at 2.73 kg per day from 100 kg until slaughter at 130 kg body weight. Pigs that were restricted in feed intake were individually fed four times daily in crates which were placed within each pen. All pigs were penned in groups of four in 1.5 x 4.6 m pens with concrete floors and straw bedding. Water was available at all times from drinking bowls. Feed intake and weight gain were recorded weekly. At 55, 100 and 130 kg liveweight blood samples were taken from the orbital sinus for determination of serum inorganic phosphate (Pi) concentration and walking condition was subjectively scored according to the following scale:

1= structurally sound

2= rotation of hind limbs, mild incoordination

3= slight but distinct lameness

4= moderate lameness

5= severe lameness

Pigs were slaughtered by stunning and exsanguination and left half carcasses were returned to the Department of Animal Science Meats Laboratory where limb bones were removed. Seven bone sites (Table II.5) which have a very high incidence of OC (Grondalen 1974b) were examined grossly for cartilage soundness and scored using the following scale:

1= cartilage surface smooth with no evidence of lesions.

2= cartilage surface smooth with mild osteochondrotic lesions of a focal nature.

3= moderate degree of grooves and (or) osteochondrotic lesions.

4= softening or erosion at the cartilage surface and (or) extensive osteochondrotic lesions.

5= severe cartilage erosion with exposure of subchondral bone and (or) severe and extensive OC.

6= separation of articular cartilage from subchondral bone, fracture of the proximal ulna or extreme thickening of the distal ulnar epiphyseal plate.

A score for the elbow joint of each animal was computed as the average of the scores for the medial humeral condyle, lateral humeral condyle, proximal ulna and proximal radius which comprise this joint. A score for the distal femur of each animal was computed as the average of the scores for the medial and lateral femoral condyles. Samples of cartilage with subchondral bone attached were fixed in 10% formalin in phosphate buffer and processed routinely (Nakano et al. 1981a). Evidence of chondrocyte necrosis, loss of safranin-O staining intensity or fibrosis was used to verify the occurence of lesions observed macroscopically. For bone bending moment determination, the fourth metacarpal from each pig was supported at each end and a force applied at the midpoint at a constant velocity of 5mm per min using an Instron Testing Machine (Model TTKH - Instron Corp., Canton, Massachusetts, U.S.A.). A recorder was used to plot force versus deflection and bending

moment was calculated as maximium force (kg) times distance between the two bone supports (cm) divided by four (Crenshaw et al. 1981a).

For ash determination 3 cm long mid-diaphysis sections of the femur were dried at 70°C for 4 d, broken, extracted with petroleum ether in a soxlet apparatus for 12 h, redried and ashed at 600°C for 6 h. Serum Pi was determined by the method of Goldenberg and Fernandez (1966). Samples of each ration were ground in a Wiley mill with a 0.42mm mesh screen before dry matter determination and chemical analysis. Ca was determined by atomic absorbtion spectrophotometry (Spectrophotometer Model 4000, Perkin Elmer Corp.) and P was determined by an autoanalyser procedure as described by the Association of Official Analytical Chemists (AOAC) (1980). Crude protein was determined by the Kjeldhal method (AOAC 1980).

Data were analysed by least squares analyses of variance (Harvey 1960). Sources of variation were diet (3), sex (2) and slaughter weight (4). Student Newman Keul's multiple range test was used for comparison of means and the incidence of osteochondrosis was compared among treatments using the Chi square test (Steel and Torrie 1980). Partial correlation coefficients were corrected for diet, sex and slaughter weight effects.

C. RESULTS AND DISCUSSION

Performance and leg soundness scores

Two boars from each of the low and high Ca-P treatments were removed from the test due to illness and severe weight loss. There was no significant effect of dietary Ca and P levels on growth performance of pigs during the grower, finisher or overall (20-100 kg) growth period (Table II.2). Average daily gain and feed/gain for the 20 to 100 kg period were almost identical for pigs fed the three diets. These results are consistent with those of Maxson and Mahan (1983) who reported that boar performance reached a maximium at 0.65-0.50% dietary Ca-P. Hickman et al. (1983) reported small increases in daily gain when 1.17-0.90% dietary Ca-P were fed to growing boars but this trend was reversed in the finishing period and overall performance was not affected. In the present study, average daily gain, average daily

feed intake and feed/gain ratio of pigs during the grower and finisher periods were very similar to NRC (1979) expected values. A Ca-P regimen similar to that recommended by NRC (1979) seems adequate for this rate of growth.

There were no significant differences in feed intake between the sexes. However, males grew more quickly in the finisher ($P<0.03$) and overall ($P<0.01$) periods and had significantly lower feed to gain ratios for grower, finisher and overall growth periods. After animals reached 100 kg bodyweight, performance criteria were highly variable and there was a marked increase in feed to gain ratios, especially for the female pigs (Table II.3). As intended, pigs fed ad libitum during the 100 to 130 kg growth period had greater feed intake ($P<0.001$) and daily gain ($P<0.001$) than those fed restricted levels. Boars had significantly lower ($P<0.03$) feed/gain ratios than had gilts. Feed/gain ratio was not significantly affected by feeding level. There were no significant ($P>0.05$) diet x sex interaction effects on average daily gain, average daily feed intake or feed/gain of pigs during the growing, finishing or overall growth period.

Leg soundness scores at 50, 100 or 130 kg body weight were not significantly affected by dietary Ca-P levels (Tables II.3 and II.4) and this is in agreement with the findings of Nimmo et al. (1980) and Kornegay and Thomas (1981) for growing and finishing pigs.

Serum Phosphate Levels

At 55 kg liveweight, serum Pi increased in response to increasing dietary Ca-P levels but there was no significant difference at 100 kg (Table II.4). A similar response to dietary Ca-P levels has been reported for grower pigs (Reinhard et al. 1976; Nimmo et al. 1981) and for 90 kg pigs (Hickman et al. 1983; Kornegay and Thomas 1981; Doige et al. 1975). There was a decrease in serum phosphate level during the finishing phase ($P<0.05$). An age related decline in serum Pi for pigs from 2 weeks to 5 months of age was also observed by Ullrey et al. (1967). Restricted feeding after 100 kg caused a reduction ($P<0.01$) in serum Pi (Table II.3) but there was a significant interaction between feeding and dietary Ca-P (Figure II.1). Dietary Ca-P level had no significant ($P>0.05$) effect on serum Pi of ad libitum fed pigs at 130 kg body weight. However, restricted intake of the two lower Ca-P diets during the 100 to

130 kg growth period reduced ($P<0.05$) serum Pi levels.

Cartilage Condition

The incidence of cartilage damage at the seven sites examined is shown in Table II.5. Most lesions observed were osteochondrotic in nature with some degree of cartilage retention in subchondral bone. The medial femoral condyle and the distal ulnar growth plate were abnormal in 80% of all animals examined. Fifty per cent of the distal humeri examined also had lesions. All other sites had a 35% incidence of cartilage damage. Severe abnormalities occurred most frequently in the medial condyle of the femur (26.5%) but were uncommon in the lateral condyle (1.1%). The medial humeral condyle and the distal ulna were also sites of severe and frequent cartilage damage. Abnormality of the lateral humeral condyle often took the form of cartilage surface grooves with macroscopically normal underlying cartilage and bone. On the proximal articular surface of the radius superficial cartilage erosion and thinning were the most frequent abnormalies.

Cartilage soundness scores of the medial humeral condyle were significantly correlated with condition of the proximal ulna ($r=0.25$, $P<0.01$) and of the proximal radius ($r=0.33$, $P<0.01$). This suggests primary involvement of local mechanical stress in the development of lesions or secondary damage due to friction between incongruent articulating surfaces. Nakano et al., (1983) also observed an association between osteochondrosis dissecans of the medial humeral condyle and cartilage thinning of the medial proximal radius.

There were significant partial correlation coefficients between leg soundness score and joint soundness scores for the distal femur ($r=0.17$, $P<0.03$), for the elbow joint ($r=0.24$, $P<0.01$) and for combined values for all bone sites ($r=0.24$, $P<0.01$), indicating some association between joint lesion severity and clinical lameness. Nakano et al. (1981a) and van der Valk et al. (1981) reported considerable agreement between the severity of osteochondrosis and lamness in swine. Partial correlation coefficients calculated between growth rate during the grower, finisher and and 20 to 100 kg growth periods and condition scores of the articular cartilages examined ranged from -0.08 to 0.08 and were not significant ($P>0.05$). This is in agreement with previously reported lack of significant correlation between growth rate and the

severity of joint lesions (Perrin et al. 1978; Fredeen and Sather 1978; Nakano et al. 1984).

A significantly greater ($P<0.05$) metacarpal bending moment and femoral ash content were obtained by increasing the Ca-P level of the diet from low to moderate. Further elevation of dietary Ca-P level yielded an additional increase in bone ash ($P<0.05$) but the increase in bending moment was not statistically significant. The data (Table II.6) suggest a diminished response of bone mineralization to the second dietary Ca-P increment as compared to the first but maximium mineralization seemed to occur at the highest Ca-P level. Nimmo et al. (1980) also observed a linear increase in bone ash in response to increasing Ca-P levels to 1.3-1.0 % while metatarsal peak force was maximised at 0.98-0.75 % Ca-P. It has been suggested that bone ash content may give an index of "skeletal development" in immature swine but that it does not give an indication of bone strength (Nimmo et al. 1981). Yet, the physiological significance of bone bending moment has not been established. In most bone flexure tests, a force is exerted at a right angle to the longitudinal axis of the diaphysis and a peak force is recorded (Crenshaw et al. 1981a). However, the magnitude and direction of biomechanical forces exerted on the joints and bones of animals during movement (Simon 1970) are not as represented in the flexure test. That elevated bone strength and walking condition of swine are not correlated is evidenced by a lack of improvement in walking condition of swine fed Ca-P levels in excess of their requirement for maximium growth rate (Nimmo et al. 1980; Kornegay and Thomas 1981). But visual assessment of structural soundness of swine is highly subjective and the lameness observed may be of complex etiology. Therefore, an evaluation of bone and joint lesions may be a more specific measure of the skeletal response to dietary Ca-P. In this regard, dietary Ca and P level had no significant effect ($P>0.05$) on the severity of lesions at any of the bone or joint sites examined. Mean scores for the distal femur, elbow joint and all sites combined were almost identical for pigs fed each diet. In addition, the incidence of lesions at each of the seven sites examined was not significantly different among the three dietary treatments ($P>0.05$). Similar findings have been reported by Duthie and Lancaster (1964). Doige et al. (1975) observed no significant effect of dietary Ca-P levels in excess of the low Ca-P levels used in

the present study on the severity of skeletal lesions when the dietary Ca to P ratio was maintained near 1.23 to 1. Grondalen (1974a) fed diets similar to the moderate and high Ca-P diets used in this study and at 90 kg observed no significant cartilage lesion or walking condition changes but he noted that bone trabeculae seemed thicker in pigs fed the high Ca-P diet. In a more recent study (Arthur et al. 1983), dietary Ca and P levels in excess of NRC (1979) recommendations were fed to gilts from weaning to 100 kg, but after three parities, articular cartilage soundness of these animals was not affected by the initial dietary treatments. Although elevated dietary Ca-P levels had a positive effect on the ash content of the femur and bending moment of the fourth metacarpal in the present study, these parameters were not significantly ($P>0.05$) correlated with lesion score at any joint site examined. Such an increase in bone mineralization probably results in an improved compressive strength of the trabeculae which are frequently fractured in advanced osteochondrosis dissecans (Perrin et al. 1978). It is apparent, however, that elevated dietary Ca-P levels did not delay or alleviate the onset of cartilage degeneration. This observation is supported by the results of Nakano et al. (1981b) which indicate that in normal and osteochondrotic joints, Ca and P concentrations are similar in subchondral bone adjacent to the usual sites of osteochondrotic lesions. Other potentially beneficial effects of increased mineralization of bone include reduced incidence of bone fractures and firm insertion of ligaments and tendons. In this study, bone fractures were confined to the proximal ulna and were related to severe OC and not to dietary Ca-P level.

Boars had significantly ($P<0.001$) more elbow joint abnormalities than gilts (Table II.6) but both sexes were highly susceptible to joint lesions. The medial humeral condyle and the proximal ulna and radius with which it articulates were all more severely damaged in boars than in gilts. Condition of the distal femur was not significantly different among the sexes. Nakano et al. (1979a) observed no significant effect of sex on cartilage condition of swine at 90 kg but differences may be greater at heavier slaughter weights. Some studies suggest that in humans (Mubarak and Carroll 1981), dogs (Olsson 1977) and rats (Kato and Onodera 1984), males are more susceptible to joint lesions than are females, but the reason for these sex

differences is not known. Metacarpal bending moment of boars and gilts were similar but boars had significantly lower ($P<0.001$) femoral ash content. Crenshaw et al. (1981b) noted a trend towards lower bone ash in boars relative to gilts without a concomitant change in bone bending moment.

Feed restriction during the 100 to 130 kg growth period had no significant ($P<0.05$) effect on cartilage condition or metacarpal bending moment but it did result in an increased ($P<0.05$) ash content of the femur (Table II.7), perhaps due to a greater age at slaughter (Lepine et al. 1985). At 100 kg bodyweight, bone ash content and bending moment were significantly lower than at 130 kg. It has been reported that swine of five months of age have lower bone strength and bone ash than those of seven months of age. (Crenshaw et al. 1981b). Because OC is a disturbance of endochondral ossification, it might be expected to increase in severity as bone growth occurs. In this regard, the 130 kg animals tended to have the highest ($P<0.05$) lesion scores for the lateral humeral condyle and the distal ulnar epiphysis. Previous studies indicate that from birth to market weight, there is a close relationship between age (or weight) and the development of OC (Grondalen 1974b; Nakano et al. 1979b).

Detailed studies of age changes in swine articular and epiphysial cartilage by Grondalen (1974b), Reiland (1978) and Nakano et al. (1979b) indicate that lesions first occur in the young growing animal. This is usually manifested both as an accumulation of hypertrophic cells which do not mature and as a failure of vascular penetration (Nakano et al. 1979b). These abnormalities result in a failure of ossification, retention of cartilage in subchondral bone and the development of bone lesions. The results of this study indicate that while bone ash content and bone strength respond positively to elevated dietary Ca-P, the incidence and severity of joint lesions are not reduced by maximising bone mineralization. Thus, the lack of vascular penetration which occurs in osteochondrosis may prevent the mineralization of osteochondrotic lesions regardless of increased Ca-P in the diet.

Table II.1 Composition of diets(%).

Diet	Grower			Finisher		
Ca/P level	Low	Moderate	High	Low	Moderate	High
Wheat	28.42	28.15	27.44	—	—	—
Barley	56.93	56.38	55.00	88.01	86.74	85.43
Soybean	11.70	11.60	12.40	9.50	9.80	10.10
Vitamin-mineral premix†	1.00	1.00	1.00	1.00	1.00	1.00
Calcium phosphate	0.57	1.21	2.42	0.20	0.88	1.65
Ground limestone	0.98	1.26	1.34	0.89	1.18	1.42
Iodized salt	0.4	0.4	0.4	0.4	0.4	0.4
Chemical analysis*						
Crude protein, %	17.0	16.7	16.7	14.4	14.6	14.7
Dry matter, %	89.0	90.1	90.4	89.4	89.3	89.9
Calcium, %	0.58	0.83	1.04	0.54	0.80	0.93
Phosphorus, %	0.50	0.63	0.82	0.45	0.60	0.74
Calculated composition*						
Calcium, %	0.60	0.81	1.05	0.50	0.72	0.94
Phosphorus, %	0.50	0.63	0.82	0.40	0.54	0.70

*As fed basis.

†Supplied the following per kg of grower diet: 120 mg zinc; 12 mg manganese; 150 mg iron; 12 mg copper; 0.10 mg selenium; 5000 IU vitamin A; 500 IU vitamin D_3; 22 IU vitamin E; 12 mg riboflavin; 46 mg niacin; 26 mg calcium pantothenate; 30 μg vitamin B_{12}; 500 mg choline. Supplied 50% of these levels per kg of finisher ration.

Table II.2 The effects of dietary Ca and P level and sex on pig performance from 20 to 100 kg

	Dietary Ca and P level					Sex			
	Low	Moderate	High	SE	Sig.†	Male	Female	SE	Sig.
No. of animals	62	64	62			92	96		
Grower Period (20-55 kg)									
Initial wt., kg	19.9	20.0	19.8	0.15	NS	19.9	19.9	0.12	NS
Final wt., kg	55.6	55.3	55.6	0.41	NS	55.8	55.2	0.34	NS
Avg. daily feed intake, kg	1.72	1.81	1.79	0.05	NS	1.76	1.79	0.04	NS
Avg. daily gain, kg	0.65	0.67	0.68	0.01	NS	0.67	0.66	0.01	NS
Feed/Gain	2.67	2.74	2.64	0.05	NS	2.61	2.74	0.04	P<0.03
Finisher Period (55-100 kg)									
Initial wt., kg	55.6	55.3	55.6	0.41	NS	55.8	55.2	0.34	NS
Final wt., kg	99.9	100.1	100.2	0.54	NS	100.6	99.6	0.44	NS
Avg. daily feed intake, kg	3.03	3.10	3.04	0.09	NS	3.08	3.03	0.07	NS
Avg. daily gain, kg	0.83	0.87	0.83	0.03	NS	0.88	0.81	0.22	P<0.03
Feed/Gain	3.68	3.60	3.65	0.05	NS	3.51	3.77	0.04	P<0.01
Overall Period (20-100 kg)									
Avg. daily feed intake, kg	2.36	2.45	2.41	0.04	NS	2.40	2.41	0.03	NS
Avg. daily gain, kg	0.74	0.76	0.75	0.01	NS	0.77	0.73	0.01	P<0.01
Feed/Gain	3.21	3.21	3.20	0.04	NS	3.10	3.31	0.03	P<0.001

†Significance of the F ratio for diet or sex.
NS, no significant difference (P>0.05).
SE, standard error of mean.

Table II.3 Effects of dietary calcium and phosphorus levels, feeding levels and sex on pig performance from 100 to 130 kg and on walking score and serum phosphate concentration at 130 kg.

	Dietary Ca and P level					Feeding system from 100 kg to 130 kg				Sex			
	Low	Moderate	High	SE	Sig.	Ad lib.	Restricted	SE	Sig.	Male	Female	SE	Sig.
No. of animals	31	31	31			46	47			45	48		
Avg. daily gain, kg	0.71	0.75	0.64	0.03	NS	0.85	0.55	0.03	P<0.001	0.73	0.66	0.03	NS
Avg. daily feed intake, kg	3.22	3.28	3.11	0.11	NS	3.76	2.65	0.09	P<0.001	3.17	3.23	0.09	NS
Feed/gain	4.69	4.48	4.96	0.23	NS	4.45	4.97	0.19	NS	4.38	5.04	0.19	P<0.03
Walking score at 130 kg†	2.11	2.44	1.97	0.21	NS	1.91	2.44	0.17	NS	2.14	2.21	0.17	NS
Serum inorganic phosphate at 130 kg, mg/100 ml	7.16	7.18	7.44	0.16	NS	7.58	6.94	0.13	P<0.01	7.30	7.22	0.13	NS

NS, no significant difference (P>0.05).
SE, standard error of mean.
†scored from 1 (normal) to 5 (very lame)

Table II.4 Effects of dietary Ca and P and sex on walking score† and serum inorganic phosphate (Pi) at 55 and 100 kg

	Ca and P level in the diet					Sex				
	Low	Moderate	High	SE	Sig.	Male	Female	SE	Sig.	Mean
Walking score at 55 kg	1.66	1.49	1.50	0.13	NS	1.48	1.62	0.11	NS	—
Walking score at 100 kg	2.01	1.82	2.01	0.18	NS	2.01	1.88	0.14	NS	—
Serum Pi, mg/100 ml										
at 55 kg	7.80a	8.24b	8.57c	0.17	$P<0.05$	8.09	8.31	0.14	NS	8.20*
at 100 kg	7.82	7.86	7.96	0.19	NS	8.02	7.74	0.16	NS	7.88*

Means for diet in the same row having different letters are significantly different ($P<0.05$).
*Significant age effects ($P<0.05$).
SE, standard error of mean.
†scored from 1 (normal) to 5 (very lame)

Table II.5 Incidence of osteochondrosis in swine

Site	Incidence of lesions, %	Incidence of severe† lesions, %
Medial femoral condyle	80.0	26.5
Lateral femoral condyle	35.1	1.1
Medial humeral condyle	50.4	16.7
Lateral humeral condyle	51.4	13.0
Distal ulna	82.7	16.1
Proximal ulna	37.8	9.7
Proximal radius	36.2	10.8

†scores of four or greater were considered severe.

Table II.6 Effects of dietary Ca and P level and sex on cartilage score†, bone ash and bending moment

	Dietary Ca and P Level					Sex			
	Low	Moderate	High	SE	Sig.	Male	Female	SE	Sig.
Medial femoral condyle	2.84	2.59	2.85	0.18	NS	2.84	2.68	0.15	NS
Lateral femoral condyle	1.44	1.42	1.43	0.08	NS	1.57	1.30	0.07	P<0.01
Distal femur	2.14	2.01	2.14	0.10	NS	2.20	1.99	0.08	NS
Medial humeral condyle	2.07	2.21	1.95	0.15	NS	2.33	1.82	0.12	P<0.01
Lateral humeral condyle	1.86	1.94	2.07	0.13	NS	1.93	1.99	0.11	NS
Proximal ulna	1.86	1.94	1.64	0.13	NS	2.08	1.54	0.11	P<0.01
Proximal radius	1.79	1.69	1.87	0.15	NS	2.02	1.55	0.12	P<0.01
Elbow joint	1.89	1.95	1.88	0.10	NS	2.09	1.72	0.08	P<0.01
Distal ulnar epiphysis	2.49	2.44	2.54	0.17	NS	2.62	2.36	0.14	NS
All sites combined	2.05	2.03	2.05	0.07	NS	2.20	1.89	0.06	P<0.001
% ash, femur	68.78a	69.43b	69.86c	0.10	P<0.05	69.13	69.58	0.08	P<0.001
MC4, bending moment (kg.cm)	128a	144b	157b	4.61	P<0.05	142	144	3.77	NS

Means for diet in the same row having different letters are significantly different, P<0.05: No significant diet x sex interaction effects (P>0.05).
NS, no significant difference, P>0.05.
SE, standard error of mean.
MC4, fourth metacarpal.
†scored from 1 (normal) to 6 (severe lesion)

Table II.7 Effects of slaughter weight and feed restriction on cartilage score†, bone ash and bending moment

	Slaughter Weight				
	130 kg-ad lib.	130 kg-restricted	100 kg	100 kg-Tetracycline	SE
Medial femoral condyle	2.92	2.87	2.90	2.34	0.21
Lateral femoral condyle	1.41	1.46	1.47	1.38	0.09
Distal femur	2.17	2.17	2.19	1.86	0.12
Medial humeral condyle	2.08	2.43	1.84	1.94	0.17
Lateral humeral condyle	2.52a	2.06b	1.91b	1.36c	0.15
Proximal ulna	1.62	1.83	1.96	1.83	0.16
Proximal radius	1.64	1.90	2.00	1.59	0.17
Elbow joint	1.97	2.06	1.93	1.68	0.12
Distal ulnar epiphysis	2.32ab	2.67ab	2.92a	2.05b	0.19
All sites combined	2.07a	2.17a	2.14a	1.79b	0.08
% ash, femur	69.48a	69.80b	69.16c	68.99c	0.11
MC4, bending moment, (kg.cm)	163a	159a	129b	121b	5.33

Means in the same row having different letters are significantly different ($P<0.05$): No significant interaction effects between slaughter weight and sex or diet ($P>0.05$).
SE, standard error of mean.
MC4, fourth metacarpal.
†scored from 1 (normal) to 6 (severe lesion)

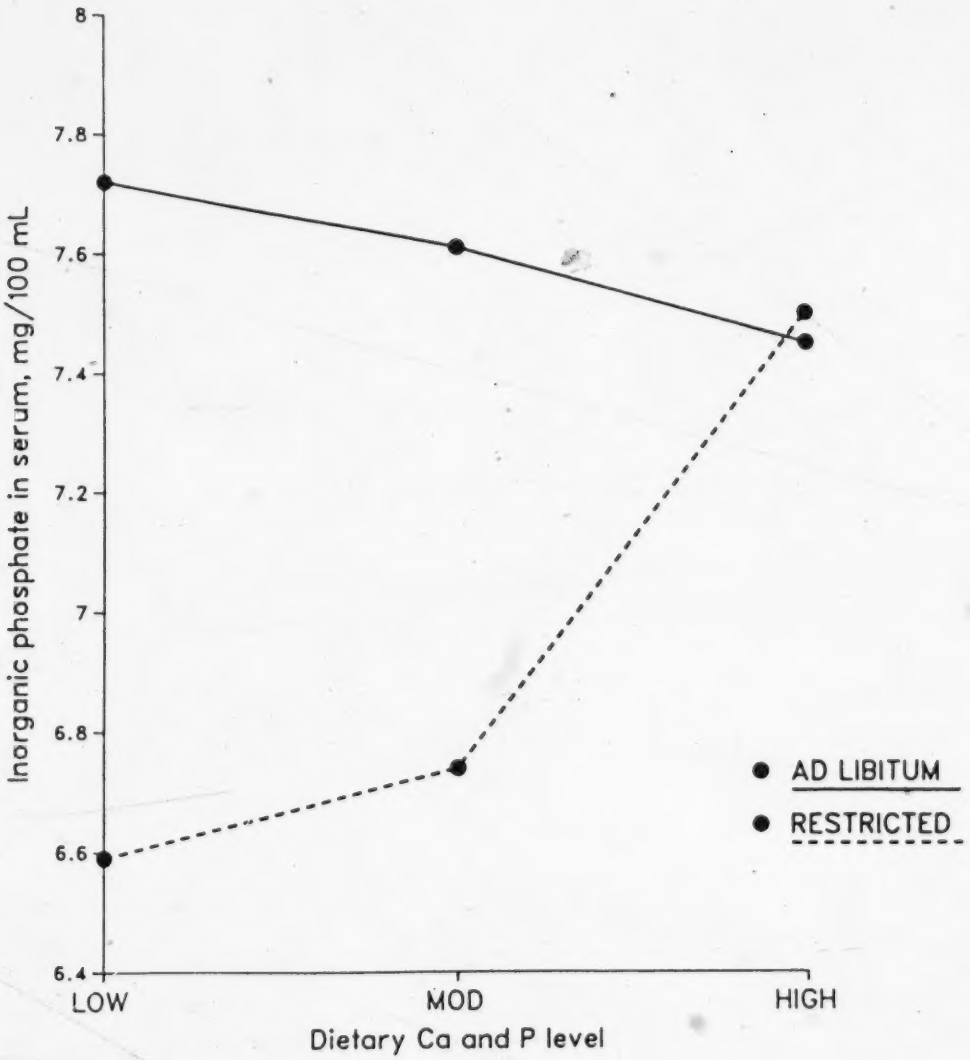

Figure II.1 Effect of feeding level from 100 to 130 kg and dietary calcium and phosphorus levels on serum inorganic phosphate concentration of swine at 130 kg bodyweight

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III. RELATIONSHIPS BETWEEN CARCASS CHARACTERISTICS, PROXIMAL PELVIC MUSCLE DISTRIBUTION AND THE SEVERITY OF OSTEOCHONDROSIS IN BOARS AND GILTS

A. INTRODUCTION

It has often been suggested that modern intensive confinement of swine in combination with increasing growth rates has aggravated the problem of osteochondrosis (OC) in swine. Grondalen (1974) demonstrated a relationship between broadness of the hams (assessed subjectively) and the severity of femoral OC. He suggested that wider hams resulted in a posture which increased the spacing between the knee joints and was associated with a sub-optimal joint-shape. In a more recent study, van der Wal et al. (1980) reported that a greater carcass length and an increased ham weight relative to side weight were positively correlated with the severity of OC of the proximal femur, suggesting the possibility of predicting the severity of OC from carcass characteristics. While the etiology of hip dysplasia in dogs is not completely understood, it has been described as a biomechanical disease resulting in part from an increase in bone growth relative to muscle growth (Riser 1974). Dogs suffering from this condition were reported to have a reduced pelvic muscle weight relative to bodyweight as compared to healthy dogs (Riser and Shirer 1967). These studies with swine and dogs imply that considerable changes in muscle distribution occur in animals suffering from bone or joint disease. Yet, total muscle dissection studies indicate that muscle distribution is relatively constant in swine and is very resistant to change (Richmond and Berg 1971; 1982).

The purpose of this investigation was to examine the relationship between carcass characteristics, proximal pelvic muscle distribution and the severity of OC in swine.

B. MATERIALS AND METHODS

The left half carcasses from each of the 192 pigs from the study described in chapter II were used to study the relationship between carcass traits and the severity of OC. Carcass length was measured from the anterior edge of the first rib to the symphysis pubis. Nine muscles from the proximal pelvic group (tensor fasia latae, biceps femoris, gluteus medius, vastus lateralis, rectus femoris, semitendinosis, gracilis, semimembranosus, and adductor femoris), the femur and the tibia were dissected and weighed. The condyles of the femur and humerus, the proximal articular cartilages of the radius and ulna and the distal ulnar growth plate were scored on a scale of one (normal cartilage) to six (severely osteochondrotic) as described in chapter II. The left half carcasses of four boars and four gilts that were slaughtered at 100 kg bodyweight and of two 130 kg boars and two 130 kg gilts from each of the restricted and ad libitum-fed treatment groups were selected for total dissection. All of these animals were fed the medium Ca/P diet described in chapter II. Half-carcasses were dissected into individual muscles, fat and bone as outlined by Butterfield and May (1965). During partial and total dissections, half-carcasses and dissected muscles were covered with moist cloths to minimise moisture loss. The tibiae and humeri from each of the 48 pigs which were treated with tetracycline hydrochloride (chapter II) were split longitudinally and examined under an ultra violet lamp. The fluorescent bands resulting from tetracycline administration at 35 and 55 kg bodyweight were visible but were too diffuse to enable precise measurements to be made. Those of the proximal humerus and proximal tibia were best defined. Therefore, the fluorescent band closest to each of the growth plates of the proximal humerus and proximal tibia was used to measure growth rate of bone during the 75 to 100 kg period using the method described by Graham and Price (1981).

Multiple regression analysis was used to determine the relationship between the mass of the selected proximal pelvic muscles and the total muscle mass in the half-carcass. The equation included sex and slaughter weight as independent variables. Proximal pelvic muscle mass was divided by the number of days on test to obtain an index of average lean tissue growth rate. Partial correlation coefficients adjusted for sex, diet and slaughter weight effects

were computed (Steel and Torrie 1980) between the severity of OC and the ratios of muscle mass contributing to anterior/posterior stability and medial/lateral stability (Table III.1) of the knee joint. Similar correlations were computed between the severity of OC and the contribution of each of the nine pelvic muscles to total proximal pelvic muscle mass. Carcass data were analysed by analysis of variance as described in chapter II.

C. RESULTS AND DISCUSSION

The effect of slaughter weight and nutritional regimens on carcass composition of boars and gilts has been well documented (Richmond and Berg 1971; 1982). Therefore, treatment means for carcass traits will not be presented in this study of OC.

The relationship between muscle distribution within a group of nine proximal pelvic muscles and cartilage soundness is shown in Table III.1. The ratios of muscles contributing to anterior/posterior stability and medial/lateral stability of the knee joint were examined because of possible influences on joint angulation. There was however, no significant association between these functional ratios and the severity of femoral OC. Apparent hypertrophy of the rectus femoris was associated with reduced OC of the medial femoral condyle. There were positive correlations between knee joint soundness scores and the relative weight of the adductor femoris which may be in agreement with the observation by Grondalen (1974) that wider hams were associated with greater knee joint damage in swine. Correlations between muscle weight ratios in the proximal pelvic group and cartilage condition in sites other than the stifle were not significant, with the exception of the distal ulnar growth plate and the medial humeral condyle, indicating a local knee-joint effect. It is not known if the apparent muscle changes preceded or resulted from joint damage but correlations computed within slaughter weight groups (Table III.2) were significant at 130 kg bodyweight but not at 100 kg, which suggests that muscle changes occured after 100 kg and may have been secondary to joint damage. Perhaps changes in animal posture due to severe joint damage or splaying of the hind legs sometimes observed in lame pigs, may alter muscle function and cause muscle hypertrophy or atrophy. It must be emphasized however, that since moisture content of

muscle and myofibre cross sectional area were not monitored in this study, the apparent changes in muscle weight may not necessarily reflect true muscle hypertrophy or atrophy.

The contributions of muscles other than the adductor femoris and rectus femoris to total proximal pelvic muscle mass were not significantly ($P>0.05$) correlated with the integrity of the medial or lateral femoral condylar cartilage. Muscle to bone ratio of the proximal pelvis, and carcass length were not significantly ($P>0.05$) correlated with cartilage damage at any bone site examined. Some previous studies (Fredeen and Sather 1978; Perrin et al. 1978) also indicate no significant association between carcass characteristics and joint damage in swine. Van der Wal et al. (1980) reported that correlation coefficients between humeral and femoral OC scores and carcass length were significant in Durocs but not in Dutch Landrace or Dutch Yorkshire pigs which suggested that a long vertebral column was associated with OC only in some breeds. These researchers also observed a significant correlation ($r=0.23$ to 0.28) between femoral OC and ham mass/side mass but in the present experiment the mass of 9 proximal pelvic muscles/side mass was not significantly ($P>0.05$) correlated with the severity of femoral OC.

The partial correlation coefficient between the mass of nine proximal pelvic muscles and total half-carcass muscle mass was 0.85 ($P<0.001$) and the multiple regression equation explained 93% of variation in total mass of side muscle. An index of lean tissue growth rate of pigs slaughtered at 100 kg bodyweight was significantly correlated with lesion score for the medial humeral condyle ($r=0.27$, $P<0.01$) but was not significantly correlated ($P>0.05$) with soundness scores for any other joint site examined. The articular cartilage of the elbow joint may be susceptible to mechanical damage, particularily in animals having high lean tissue growth rates. Nevertheless, variation in lean tissue growth rate of the magnitude observed in this study cannot be regarded as an important factor in the etiology of OC because it explained less than 8% of variation in lesion score at one cartilage site. In addition, there was no significant ($P>0.05$) correlation between growth rate at the proximal epiphysis of the humerus or tibia during the 75 to 100 kg growth period and the severity of OC at any joint site examined.

The results indicate that carcass characteristics and growth rate of lean tissue and bone are poor predictors of OC in swine, but that knee joint damage is associated with some local muscle distribution changes.

Table III.1 Partial correlation coefficients between cartilage soundness score and proximal pelvic muscle distribution

	Anterior/† Posterior†	Medial/‡ Lateral‡	Rectus Femoris/ Total[1]	Adductor Femoris/ Total	Adductor Femoris/ Rectus Femoris
Medial F. Condyle	0.13	-0.03	-0.19*	0.17*	0.23*
Lateral F. Condyle	0.06	0.00	-0.03	0.22**	0.21**
Distal Femur	0.14	0.03	-0.18*	0.23**	0.29***
Medial Humerus	0.06	-0.00	-0.12	0.23**	0.26***
Lateral Humerus	0.01	0.16*	-0.03	-0.17*	-0.13
Prox. Ulna	-0.02	-0.03	-0.05	0.07	0.09
Prox. Radius	0.09	0.08	-0.08	0.03	0.07
Elbow Joint	0.06	0.10	-0.13	0.08	0.14
Distal Ulna	0.17*	0.05	-0.10	0.17*	0.20**
All sites combined	0.14*	0.08	-0.20**	0.21**	0.29***

Significance of correlation coefficient; * $P<0.05$, ** $P<0.01$, *** $P<0.001$.
†(Biceps Femoris + Tensor Fascia Latae + Gracilis + Semimembranosus)/(Vastus Lateralis + Rectus Femoris)
‡Semimembranosus/Biceps Femoris.
[1]Total mass of nine proximal pelvic muscles.

Table III.2 Partial correlation coefficients between distal femoral lesion score and proximal pelvic muscle mass ratios

Slaughter weight	Rectus Femoris/Total	Adductor Femoris/Total†	Adductor Femoris/Rectus Femoris
100 kg	-0.13 (NS)‡	0.17 (NS)	0.20 (NS)
130 kg	-0.20 (NS)	0.28 ($P<0.01$)	0.36 ($P<0.001$)

†Total mass of nine proximal pelvic muscles
‡Values in parenthesis represent significance of correlation coefficient; NS, $P>0.05$.

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IV. EFFECTS OF FLOOR TYPE ON THE SEVERITY OF FOOT LESIONS AND OSTEOCHONDROSIS IN SWINE

A. INTRODUCTION

The leg weakness syndrome is a serious problem of modern intensive swine production systems. A number of studies indicate that osteochondrosis which is manifested as a disturbance of endochondral ossification, contributes significantly to this problem (Nakano et al. 1981; van der Valk et al. 1981). Also of concern in recent years is an increase in the incidence and severity of foot lesions in swine which may also contribute to locomotory problems. Penny et al. (1963) carried out a detailed survey to assess the frequency of foot lesions in slaughter weight hogs. In the 3,195 pigs examined, the incidence of claw lesions was 65%. While all lesions do not result in lameness, those wounds or abrasions which penetrate the pain sensitive corium do cause pain and possibly result in infection and subsequent lameness (Geyer and Gloor 1985).

Some studies indicate that, of the slatted floors available commercially, plastic coated expanded metal is less likely to cause foot lesions in swine. Sows have better traction on this material than on slats of perforated metal, fiberglass or concrete (Christison and Bergeron 1984); it is most preferred by newborn and weaner pigs while woven wire is selected least (Farmer and Christison 1982). Kornegay et al. (1981) reported that plastic coated expanded metal caused less severe foot lesions in starter pigs than did a woven wire floor. Several reports have suggested that foot lesions do not reduce growth rate or feed conversion efficiency of starter (Kornegay et al. 1981) or feeder (Wright et al. 1972; Fritschen et al. 1976) pigs. These lesions may however cause changes in the posture of the pig which result in weight being borne by regions of the knee and elbow joint cartilage which are normally non weight-bearing. In this regard, Smith and Robertson (1971) noted that severe foot damage in sows was accompanied by flexing of the hock and tarsal joints. It is not known however if such postural changes adversely affect articular cartilage.

The objective of this experiment therefore was to study the effect of floor type on the severity of foot lesions in starter and market-weight hogs and to investigate the relationship between such lesions and the severity of osteochondrosis.

B. MATERIALS AND METHODS

Sixty-seven pigs were weaned at five weeks of age and randomly assigned to pens with plastic coated expanded metal or woven wire flooring. Sex was disregarded in the allocation of pigs to treatments. The pens provided .36m^2 of floor space per pig. A 20% crude protein diet was fed ad libitum and pig weight was recorded weekly. The feet of each pig were examined at weaning and at the end of the 28 day test period for overall soundness; each foot was scored on a scale from one (normal) to five (severe lesion) and an average foot pad score was computed for each pig. At the end of the 28 day test period 11 gilts which had been on plastic coated flooring and 12 gilts which had been on woven wire flooring were moved to individual 1.2 x 1.2m pens with the similar floor types. These pigs were fed ad libitum a 16% crude protein diet from 22 kg to 55 kg bodyweight and a 14% crude protein diet from 55 kg until slaughter at 94 kg bodyweight. The gilts were slaughtered by stunning and exsanguination. Feet which were representative of a normal or severely damaged condition were removed, perfused with and stored in 10% formalin in phosphate buffer, pH 7.0. Limb bones were removed and frozen for gross evaluation. The condyles of the femur and humerus, the articular cartilage of the proximal ulna and radius, and the distal epiphyseal plate of the ulna were each scored on a scale of one (normal) to five (severe osteochondrosis). For each foot the inner and outer toe, hoof wall, bulb and assessory digit were each subjectively scored on a scale of one (normal) to three (severe lesion). In addition, sections were taken through areas of erosion of the bulb and the toe of each perfused claw and soaked for four days in a solution (4.0g mercuric chloride, 0.5g chromic acid, 10ml conc. nitric acid, 50ml 95% ethanol and 200ml distilled water) to soften the keratin. Tissues were then embedded in paraffin wax and cut at at 6μm and stained with hematoxylin and eosin.

C. RESULTS

At weaning, the incidence of foot lesions was only 7%. During the starter period, floor type did not have any significant ($P>0.05$) effect on growth rate but foot pad lesions were much more severe ($P<0.01$) in pigs which were penned on woven wire flooring (Table IV.1). Average daily gain, average daily feed intake and feed/gain were similar among floor types during the grower, finisher and overall growth periods (Table IV.2). At 94 kg bodyweight, scores for the toe, bulb, hoof wall and assessory digit of both hind and fore feet were slightly higher for gilts which were penned on the woven wire floor (Table IV.3) but only in the hind toe did this difference approach statistical significance ($P<0.06$). With the exception of the front toes and the hind assessory digits, the outer digits of both front and hind feet had more severe lesions ($P<0.01$) than the inner digits at each of the four sites examined (Table IV.4). Cartilage soundness scores for the condyles of the femur and humerus, the articular cartilage of the proximal ulna and radius, and the distal epiphyseal plate of the ulna were not significantly affected ($P>0.05$) by floor type (Table IV.5). There were significant correlation coefficients between condition of the proximal radius and foot lesion scores for the toe and hoof wall of the front foot but no such relationship was evident for the humeral condyles (Table IV.6). Also, there were no significant correlations between foot lesion scores for the hind limbs and cartilage condition scores of the medial or lateral femoral condyles.

In all claws examined histologically there was severe irregularity of the volar surface of the toe and bulb due to superficial erosion of the stratum corneum, with a few clefts extending deeply into the stratum corneum. In one sample, adjacent to such a deep cleft, there was moderately severe infiltration of the dermal lamellae and the adjacent epidermis by neutrophils and to a lesser extent by mononuclear cells.

D. DISCUSSION

There was a very low incidence of foot lesions (7%) in pigs weaned at four weeks of age. Nursing pigs were not included in the present study, but previous reports indicate a sharp increase in the incidence and severity of bruising, erosion and swelling of the assessory digits and foot pads during the first week of life followed by rapid healing during subsequent weeks (Smith and Mitchell 1976; Svendsen et al. 1979). It has been suggested that a backward thrusting motion of the legs during suckling was primarily responsible for foot and skin lesions in baby pigs (Smith and Mitchell 1976) but these injuries seem to be of minor longterm significance with 96% of pigs affected at 3 days of age and only 12% of pigs affected at 3 weeks (Svendsen et al. 1979). The greater severity ($P<0.01$) of foot lesions in starter pigs which were penned on woven wire flooring and the relatively mild lesions which were associated with plastic coated expanded metal flooring might indicate that woven wire is less than ideal as a flooring material for starter pigs. Yet, average daily gain was not significantly ($P>0.05$) affected by floor type and there was no evidence of pig lameness during the starter period. Kornegay et al. (1981) observed a similar effect of these floor types on foot soundness scores of starter pigs but growth rate was not depressed by the apparent foot injuries. Because of the unevenness of the surface of woven wire, it may have provided a smaller contact area with the foot pad and therefore resulted in higher pressures on the pad and hoof wall. The response to increased pressure may include increased keratinization of the pad, but apparent lesions which are confined to the keratin layer are unlikely to cause infection or pain. Keratinisation of the pads seemed to increase in all pigs between weaning and 22 kg bodyweight.

At slaughter weight (94 kg), soundness scores were slightly higher for gilts which were penned on the woven wire floor but this difference approached significance ($P<0.06$) only in the hind toe. This is partly due to the small number of animals used in the study but the magnitude of the difference in foot scores due to floor type was much smaller at 94 kg than at the end of the starter period. Such a trend may be indicative of an adaptation of the foot to the demands of the floor type which was introduced at weaning. Fritschen and

Cunningham (1974) reported that feeder pigs adapted to clay floors by a softening of the bulb while concrete floors stimulated bulb hardening.

In market-weight gilts, lesions on the outer aspect of the foot were more severe than those on the inner aspect ($P<0.01$). Previous reports also indicate that the outer digits have the highest incidence of lesions and that they are most susceptible to injury (Penny et al. 1963; Calabotta et al. 1982). In swine, the outer claws are wider (1.13:1) and longer (1.11:1) than the inner claws (Penny et al. 1963) and a recent study of foot pressure patterns in swine of 9 kg to 107 kg bodyweight indicated that most weight was placed on the outer bulb while the weight carried by the hoof wall was usually less than 30% of bulb load (Webb 1984). Fritschen (1980) reported that the area of the claw remained nearly constant during the finishing period (55 to 100 kg) despite a doubling of body mass. But compression and deformation of the bulb and hoof wall increased the contact area and maintained constant foot pressures over a wide range of body weights (Webb 1984). Therefore, foot pressure per se cannot explain the increasing severity of foot lesions with aging but deformation or trauma might play a significant role. It is apparent however that floors with large void spaces greatly increase pressure on the feet of pigs of all ages.

Cartilage condition scores for each of the bone or joint sites examined were not significantly ($P>0.05$) affected by floor type. Perrin et al. (1978) reported that the incidence and severity of cartilage lesions in swine which were serially slaughtered between 20 and 120 kg bodyweight, were not significantly affected by housing on concrete versus earthen flooring. The influence of floor type on the locomotory system seems therefore, to be confined to local foot abrasions and does not seem to extend to the knee and elbow joints. This conclusion is supported by the low correlation coefficients between cartilage and foot soundness scores. In addition, the etiology of foot lesions seems to differ fundamentally from that of osteochondrosis in that foot lesions of baby pigs are largely reversible and growth of the hoof and pad and concomitant wear of the keratinized surface layer provide a mechanism for the replacement of damaged foot tissues. Histological investigations suggest that in market-weight hogs, many lesions are confined to the keratinized layer of the bulb and do not penetrate the

corium.

Table IV.1 Effect of floor type on performance criteria and foot pad scores of starter pigs

	Plastic-coated	Wire Mesh	Significance
Initial weight (kg)	8.19 (.29)†	8.77 (.31)	NS
Final weight (kg)	22.3 (.42)	22.7 (.46)	NS
Daily gain (kg)	0.51 (.01)	0.50 (.01)	NS
Mean pad score‡	1.62 (.09)	2.43 (.10)	$P<0.01$
Number of animals	36	31	

NS, no significant difference, $P>0.05$.
†Values in parenthesis represent standard error of mean.
‡Scored on a scale of 1 (normal) to 5 (severe lesions).

Table IV.2 Effect of floor type on performance criteria of gilts during the grower, finisher and overall growth period

	Plastic-coated	Wire Mesh	Significance
Grower Period			
Initial weight (kg)	22.3 (.47)†	22.5 (.45)	NS
Final weight (kg)	55.3 (.26)	54.8 (.24)	NS
Daily gain (kg)	0.68 (.01)	0.71 (.01)	NS
Daily feed (kg)	1.71 (.05)	1.77 (.03)	NS
Feed/Gain	2.50 (.04)	2.48 (.04)	NS
Finisher Period			
Initial weight (kg)	55.3 (.26)	54.8 (.24)	NS
Final weight (kg)	93.1 (.83)	93.9 (.79)	NS
Daily gain (kg)	0.83 (.03)	0.75 (.03)	NS
Daily feed (kg)	2.46 (.07)	2.26 (.07)	NS
Feed/Gain	2.98 (.08)	3.03 (.08)	NS
Overall Growth Period (20-94 kg)			
Daily gain (kg)	0.75 (.02)	0.73 (.02)	NS
Daily feed (kg)	2.07 (.04)	2.03 (.04)	NS
Feed/Gain	2.76 (.08)	2.78 (.08)	NS
Number of animals	11	12	

NS, no significant difference, $P>0.05$.
†Values in parenthesis represent standard error of mean.

Table IV.3 Effect of floor type on foot soundness scores† of 94 kg gilts

Site	Floor Type		
	Plastic-coated	Woven Wire	Significance
Hind Feet			
Toe	1.13 (.09)‡	1.38 (.09)	P<0.06
Pad	1.69 (.13)	1.83 (.12)	NS
Hoof wall	1.35 (.13)	1.59 (.13)	NS
Assessory digit	1.27 (.15)	1.59 (.15)	NS
Front Feet			
Toe	1.31 (.22)	1.78 (.21)	NS
Pad	2.21 (.18)	2.24 (.18)	NS
Hoof wall	1.63 (.16)	1.71 (.15)	NS
Assessory digit	1.48 (.12)	1.52 (.11)	NS

†Scored on a scale of 1 (normal) to 3 (severe lesion).
‡Values in parenthesis represent standard error of mean.
NS, no significant difference, P>0.05.

Table IV.4 Soundness scores† for the inner and outer aspects of the feet of 94 kg gilts

	Aspect			
	Outside	Inside	SE	Significance
Hind Feet				
Toe	1.40	1.11	.07	P<0.01
Pad	2.11	1.42	.07	P<0.01
Hoof wall	1.76	1.20	.11	P<0.01
Assessory digit	1.53	1.35	.08	NS
Front Feet				
Toe	1.64	1.47	.10	NS
Pad	2.60	1.85	.13	P<0.01
Hoof wall	2.04	1.29	.16	P<0.01
Assessory digit	1.80	1.20	.12	P<0.01

†Scored on a scale from 1 (normal) to 3 (severe lesion).
SE, standard error of mean.
NS, no significant difference, P>0.05.

Table IV.5 Effect of floor type on cartilage soundness scores of 94 kg gilts

Site	Floor Type		Significance
	Plastic-coated	Woven Wire	
Medial femoral condyle	2.41 (.31)‡	2.33 (.30)	NS
Lateral femoral condyle	1.00 (.04)	1.08 (.04)	NS
Medial humeral condyle	1.27 (.14)	1.17 (.13)	NS
Lateral humeral condyle	1.36 (.24)	1.50 (.23)	NS
Proximal ulna	1.23 (.23)	1.58 (.22)	NS
Distal ulna	2.84 (.20)	2.46 (.19)	NS
Proximal radius	1.09 (.19)	1.54 (.18)	NS

NS, no significant difference, $P>0.05$.
†Scored on a scale from 1 (normal) to 5 (severe lesion).
‡Values in parenthesis represent standard error of mean.

Table IV.6 Correlation coefficients between cartilage condition and soundness scores of the fore-legs of gilts

Site	Medial Humerus	Proximal Radius
Toe	.09	.64**
Pad	.01	-.15
Hoof wall	-.11	.37*
Assessory digit	-.25	-.23

*P<0.05
**P<0.01

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V. EFFECTS OF INCREASED WEIGHT STRESS ON JOINT INTEGRITY AND CATHEPSIN B-LIKE ACTIVITY OF ARTICULAR CARTILAGE IN GROWING SWINE [1]

A. INTRODUCTION

Osteochondrosis is a common disease of the growing skeleton in many species, including humans (Mubarak and Carroll 1981) and swine (Nakano et al. 1979b). The incidence of non-infectious joint degeneration in adolescent commercial pigs is almost 100%: the medial condyles of the femur and humerus being the most common sites of joint degeneration (Nakano et al. 1984). Osteochondrosis dissecans, which is characterised by cartilage detachment from subchondral bone, occurs most frequently on the weight-bearing surface of the medial humeral condyle (Nakano et al. 1983) and may be partly caused by joint overloading. The prevalence of lesions on the weight-bearing regions of articular cartilage and the tendency of articular cartilage to be flatter in osteochondrotic than in normal femoral condyles (Nakano et al. 1979a; Nakano et al. 1979b) suggests secondary, if not primary involvement of mechanical stress in the development of osteochondrosis.

It has been established that decreased weight-bearing (Palmoski et al. 1980) or severe overloading (Dekel and Weissman 1978) of joints causes articular cartilage degeneration. But between these two extremes lies a range of physiological loads which may alter the biochemical composition of cartilage while the tissue remains histologically normal. Such physiological loading increases articular cartilage proteoglycan (PG) content in some species (Catherson and Lowther 1978; Tammi et al. 1983), and it may even play a role in the prevention of joint degeneration. The PGs of articular cartilage which remain expanded due to their polyanionic nature and most of which form aggregates with hyaluronic acid in the cartilage, bind a large volume of water, giving the tissue its resilience and its ability to support increased weight for short periods of time (Maroudas 1979). Therefore, mechanically induced changes of a focal or extensive nature in cartilage PG content or aggregate size, could facilitate or prevent the development of cartilage degeneration. Changes in cartilage PG content may result from

[1] A version of this chapter has been accepted for publication. 1985. Proc. Swine in Biomed. Res. Conf. Brennan, J.J., Aherne, F.X., Thompson, J.R. and Nakano, T.

altered rates of PG synthesis or degradation. It has been suggested that mechanical overloading of joint cartilage may stimulate synthesis, release, or activation of lysosomal enzymes which degrade PGs and further increase tissue susceptibility to mechanical damage (Ali and Evans 1973). There is little information available concerning the hydrolytic enzymes responsible for PG degradation or the response of swine articular cartilage to mild mechanical stress in vivo. The objective of this experiment was to determine the response of articular cartilage and synovial fluid of immature swine to a small increase in joint load.

B. MATERIALS AND METHODS

Twenty female pigs (20.5 kg) were randomly assigned to a mechanical stress or control treatment. The pigs were penned individually in 1.2 x 1.5 m pens with wire mesh flooring, and fed a 20% crude protein diet ad libitum. The temperature of the barn was maintained at 22°C. Mechanical stress was achieved by placing an initial load of 15% of body weight in shoulder bags on the animals. This ratio was maintained by weekly increases in load weight. Control animals were not loaded, but were otherwise treated the same as the mechanically stressed group. Feed intake and pig weight were recorded weekly. Eight gilts from each treatment were observed from 1000 to 1230 h and from 1330 to 1600 h on each of days 6, 11, and 16 to estimate the amount of time spent standing or walking. The observer was visible to the animals and the position of the animals was recorded at 2 min intervals.

After a 27 day test period, pigs were sacrificed and limb bones were removed with elbow and knee joints intact. A 1 cm incision was made on the medial side of the elbow joint bursa. A 0.7 cm^2 piece of Whatman #41 filter paper was inserted between the humeral condyles and the proximal ulna using a forceps. After flexing the joint, the fluid soaked paper was removed, weighed, and used in the determination of hyaluronic acid concentration by the method of Jourdian et al. (1979) using hyaluronic acid from human umbilical cord as standard (Sigma Chemical Co., St. Louis, Missouri).

Elbow joints were opened and washed with cold distilled water. One transverse strip of cartilage of approximately 2mm x 10mm was taken from the weight-bearing region of each

of the left and right medial humeral condyles and fixed in 10% formalin in buffered phosphate, pH 7.0. The remaining condylar cartilage was diced (1 to 2 mm^2) at 4°C in distilled water, blotted dry and samples weighing approximately 200 mg were frozen in 500 μL of water in cylindrical blocks (Ali et al. 1967). These were mounted and sectioned at 18 μm on a cryostat at -20°C cabinet temperature. Sections were collected, suspended in 2.5 mL of 87 mM formate/acetate/phosphate buffer, frozen and thawed, and centrifuged at 4°C. Cathepsin B-like activity of the supernatant (cartilage extract) was determined by the method of Barrett (1976). In preliminary studies, benzoyl-DL-arginine-2-naphthylamide (BANA) was used as substrate, but it was relatively insensitive. Therefore, benzyloxycarbonyl-arginine-arginine-4 methoxy-2-naphthylamide (Enzyme Systems Products, Livermore, California) (Z-Arg-Arg-MNA) was used routinely as substrate at a final concentration of 2 mM (McDonald and Ellis 1975). To determine optimium pH for the assay of cathepsin B-like activity in swine articular cartilage, tissue slices were suspended in 87 mM formate/acetate/phosphate buffer of pH 4.0, 5.0, 5.5, 6.0, or 7.0 for 2 h and cathepsin B-like activity of the supernatants was determined. In addition, cartilage extracts were pre-incubated for 10 min at 37°C in buffer (pH 5.2) containing 2 mM ethylenediamine tetraacetate (EDTA) alone, containing both 2 mM EDTA and 2 mM cysteine, or were pre-heated for 15 min at 90°C in buffer containing 2 mM EDTA and 2 mM cysteine (Fig. V.3). Substrate (2 mM final concentration) was added at the end of the pre-incubation period and 500 μL aliquots were removed from the media at 2 h intervals for β-naphthylamide determination. On the basis of these studies, the optimal conditions were pH 5.2 and an incubation period of 2 h.

After acetone drying (Brandt and Muir 1969), condylar cartilage samples of 20 to 25 mg dry weight were digested with papain (5 mg of twice crystalized enzyme per g dry tissue) for 48 h at 60°C (McDevitt and Muir 1976). The digests were centrifuged and the supernatants were diluted to 50 mL with 0.15 M sodium acetate buffer (pH 6.8) and the uronic acid content of 0.5 mL aliquots was determined by the carbazole reaction (Bitter and Muir 1962).

Seven micron thick sections of the cartilage fixed in 10% formalin were cut perpendicular to the surface of the medial humeral condyle and stained with safranin-O, fast green, and iron hematoxylin (Lillie 1965) for histological study.

Behavioural data were transformed using the inverse sine transformation and analysed by analysis of variance of repeated measures (Steel and Torrie 1980). Sources of variation were treatment and day of observation. The total daily 5 h observation period was considered the experimental unit. Other parameters were analysed by a oneway analysis of variance.

C. RESULTS

All animals remained healthy and structurally sound throughout the experimental period. Average daily gain and average daily feed intake were not significantly ($P>0.05$) affected by mechanical loading (Table V.1). Control animals spent an estimated 40% more time standing or walking than did the weighted animals, but this difference was not significant due to large within-treatment variation. Since inverse sine transformation did not change the interpretation of the data, only untransformed means are presented.

Upon visual examination, the articular cartilage and synovial fluid of all elbow joints appeared normal. Histological examination of medial humeral condylar cartilage revealed osteochondrotic lesions in both control and mechanically stressed groups. Lesions associated with a reduced proteoglycan staining occurred at the articular surface of the medial humeral condyle (Fig. V.1) of 50% of the loaded animals but were not observed in in any of the control animals. In addition, cell clustering and loss of proteoglycan in the proliferative zone sometimes accompanied the surface lesions.

Elbow joints contained only sufficient synovial fluid to form a film over the articular surfaces and soft tissues of the joint. None of the synovial fluid samples were hemorrhagic and the hyaluronic acid concentrations did not differ significantly among treatments (Table V.2).

Hydrolysis of Z-Arg-Arg-MNA by articular cartilage extract was maximal when the final pH of the incubation medium was approximately 5.2 (Fig. V.2). This activity was linear

over a 4 h incubation period and was almost completely destroyed by preheating the cartilage extract at 90°C for 15 minutes (Fig. V.3). Cartilage cathepsin B-like activity was not significantly affected ($P>0.05$) by joint loading. Uronic acid content was similar among control and loaded animals, Table V.2.

D. DISCUSSION

The ratio of load weight to bodyweight was maintained at 15% throughout the experiment but the frequency and duration of activity and hence weight-bearing by loaded animals varied widely, giving a heterogenous treatment to the joint.

The gross integrity of distal humeral cartilage and subchondral bone was consistent with the observed structural soundness of all animals in this study, but histological observation indicated possible long term adverse effects of joint loading on articular cartilage and on the ossification process. Articular surface lesions associated with a loss of safranin-O staining were observed only in the weight bearing region of the medial humeral condyle of mechanically stressed pigs, which suggests that these lesions were caused by joint overloading. The reduction in safranin-O staining intensity occurred in areas adjacent to small cartilage fissures but it is not known if PG loss preceded cartilage microfracture. In some loaded animals cell clustering was observed in the proliferative zone, directly proximal to the articular surface lesions, a pattern which may indicate involvement of mechanical stress in the development of this abnormality. Cell clustering has been reported in osteochondrotic cartilage (Nakano et al. 1979b) and it is a potential cause of ossification failure. Apart from the fore-mentioned lesions, the articular cartilage of control and loaded animals stained intensely with safranin-O, had similar concentrations of uronic acid, and had apparently normal chondrocytes. These observations suggest that loading did not cause an extensive catabolic response in cartilage. The uronic acid concentration determined (7.0%) is similar to a previously reported value for swine of 10 weeks of age (Nakano et al. 1979b). However, Simunek and Muir (1972) reported that swine articular cartilage contained only 3.0% uronic acid at this age.

Several studies indicate that articular cartilage is very responsive to changes in load. Continuous mechanical pressure reduces PG turnover of articular cartilage in vitro (Jones et al. 1982). Similarly, cyclic stress of long cycle duration reduces net glycosaminoglycan synthesis in canine femoral condylar cartilage plugs (Palmoski and Brandt 1984), but a cyclic stress of short duration has an opposite effect. These reports indicate that chondrocyte metabolism is strongly influenced by the frequency and duration of cartilage compression. Articular cartilage of rabbit (Tammi et al. 1983) and sheep (Catherson and Lowther 1978) joints which were overloaded for four weeks by immobilization of the contralateral limb, had higher concentrations of PGs than cartilage from control joints, which is indicative of a favourable adaptation to joint loading in these species. When canine knee joints were overloaded for 4 to 8 weeks using this technique, the condylar cartilage was histologically and grossly normal (Palmoski et al. 1979; Palmoski et al. 1980). Paatsama et al. (1975) also found no evidence of degeneration in immature proximal humeral articular cartilage of dogs which had been mechanically stressed for 4 months. In the present study however, loading caused focal degeneration of the articular cartilage, but a generalized loss of PGs did not occur. The development of articular surface lesions in loaded joints may have been due to increased shear stress at the surface of the medial humeral condyle while cell clustering in the middle and deep zones may coincide with points of maximum mechanical load. It has been suggested that fast growth rate (Goedegebuure et al. 1980) and increased muscle mass (Kincaid and Lidvall 1983) have placed excessive stress on some diarthrodial joints of immature swine. Therefore, it is possible that normal articular cartilage of rapidly growing swine is at the limit of its weight-bearing capacity and even a small increase in joint loading results in cartilage degeneration. Lesions, similar to those reported herein, have been induced by mechanical overuse and simultaneous overloading of rabbit knee joints (Dekel and Weissman 1978) or prolonged treadmill exercise of dogs (Vasan 1983), but the magnitude and duration of mechanical stress employed was much greater than in the present study. Previous reports indicate that even a short period (2-4 weeks) of overloading of the elbow joint in growing swine causes osteochondrosis of the distal epiphyseal plate of the ulna (Walker at al. 1966;

Grondalen and Grondalen 1974), which is probably due to a disruption of metaphyseal blood flow (Grondalen and Grondalen 1974). Our results suggest that articular cartilage lesions may also be mechanically induced, indicating a considerable susceptibility of pigs to mechanically induced osteochondrosis.

Synovial fluid functions in the lubrication and nutrition of articular cartilage. Hyaluronic acid in synovial fluid is an important soft tissue lubricant (Swann et al. 1974), and its concentration is often reduced in humans with rheumatoid arthritis (Balazs et al. 1967). Since concentrations of this glycosaminoglycan were similar in synovial fluid of control and loaded animals, and the fluid was not hemorrhagic, it appears that the synovial membrane was not significantly damaged by joint overloading and synovial fluid dilution did not contribute to erosion of the articular cartilage surface observed in the mechanically stressed animals.

Lysosomal enzymes are usually located within the lysosome but if released into the extracellular matrix, they may cause matrix degradation. Cathepsin B degrades PGs from human cartilage (Morrison et al. 1973) and since the pH of extracellular fluid in proximity to cartilage PG may be quite low (Poole and Mort 1981), this enzyme may play a role in PG degradation in healthy and diseased cartilage. Z-Arg-Arg-MNA is susceptible to hydrolysis by cathepsin L as well as by cathepsin B (Katunuma et al. 1983), but it is more sensitive to the latter enzyme. The thiol dependence of the enzyme activity and the enhancement of activity by EDTA is in agreement with the properties of cathepsin B as described by Barrett (1972). Joint overloading did not result in a significant change in cathepsin B-like activity in humeral condylar cartilage, indicating that a generalized increase in activity of this thiol proteinase did not occur in the weight-bearing regions of the articular cartilage. Possible localized increases in activity associated with sites of focal degeneration could not be detected in the large cartilage samples used. Bayliss and Ali (1978) reported cathepsin B-like activity in normal adult human articular cartilage which is approximately 23 times less than the present value for immature swine articular cartilage, but this is probably due to the lower sensitivity of the substrate used (BANA) (Recklies et al. 1980).

In conclusion, mild mechanical stress did not change the uronic acid content or cathepsin B-like activity of articular cartilage in young swine but loading did cause focal degenerative changes which could predispose these animals to osteoarthrosis and osteochondrosis at maturity.

Table V.1 Effect of loading on performance criteria and behaviour of gilts

	Treatment			
	Loaded	Control	SE	Significance
Initial wt. (kg)	20.4	20.6	0.36	NS
Final wt. (kg)	42.4	42.7	1.02	NS
Average daily gain (kg)	0.82	0.82	0.03	NS
Average daily feed inake (kg)	1.48	1.59	0.04	NS
Feed conversion efficiency	1.81	1.93	0.05	NS
% of time spent standing or walking	12.8	17.0	2.90	NS

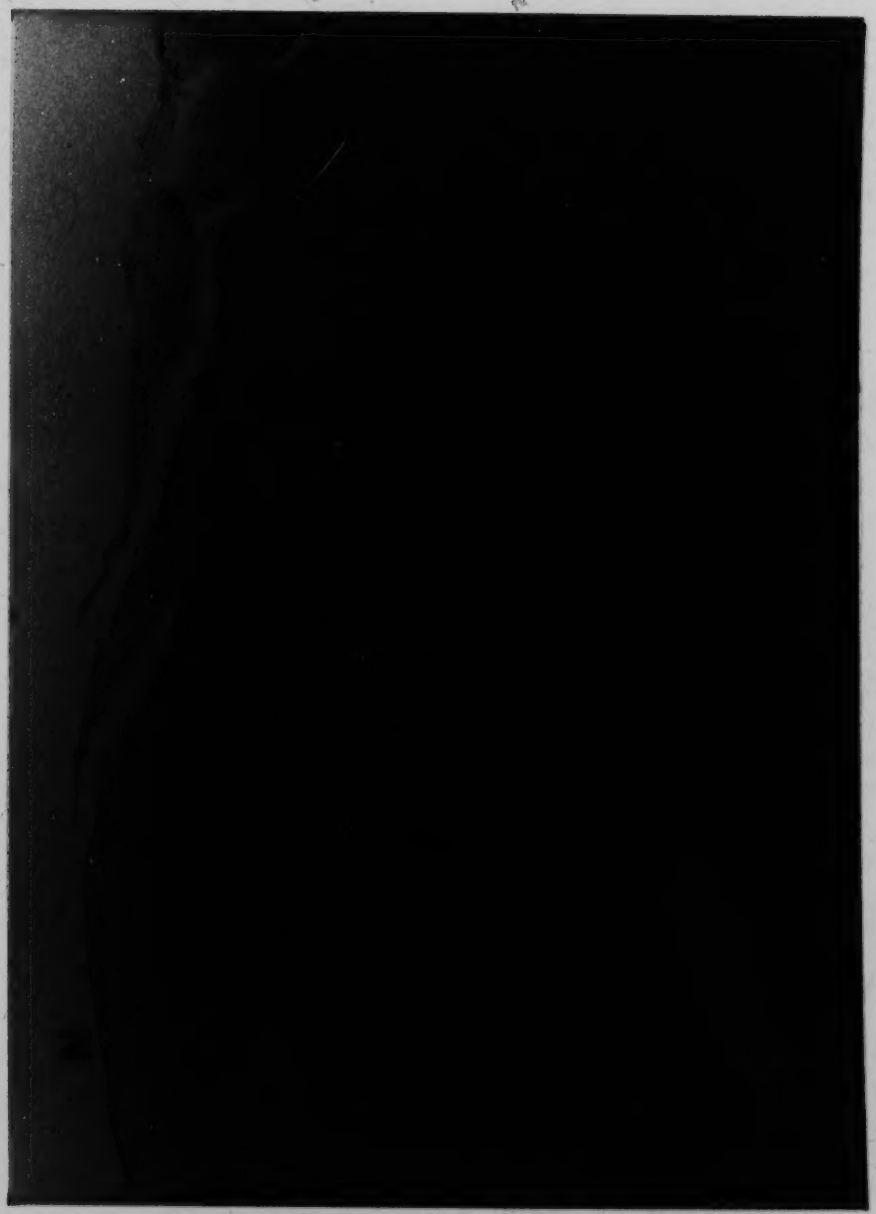
NS, means not significantly different ($P>0.05$).
SE, standard error of mean.

Table V.2 Effect of loading on synovial fluid hyaluronic acid concentration, articular cartilage cathepsin B-like activity and uronic acid content

	Treatment			
	Loaded	Control	SE	Significance
Elbow joint synovial fluid hyaluronate (mg/ml)	2.42	2.58	0.14	NS
Cathepsin B-like activity, μmoles of substrate hydrolysed/(h.g of dry cartilage)	2.25	2.56	0.14	NS
Uronic acid, % of cartilage dry weight	6.92	7.10	0.14	NS

NS, means not significantly different ($P>0.05$).
SE, standard error of mean.

Figure V.1 Articular surface lesion (arrow) of the medial humeral condyle with reduced proteoglycan staining intensity adjacent to an area of normal intact articular cartilage surface (N).



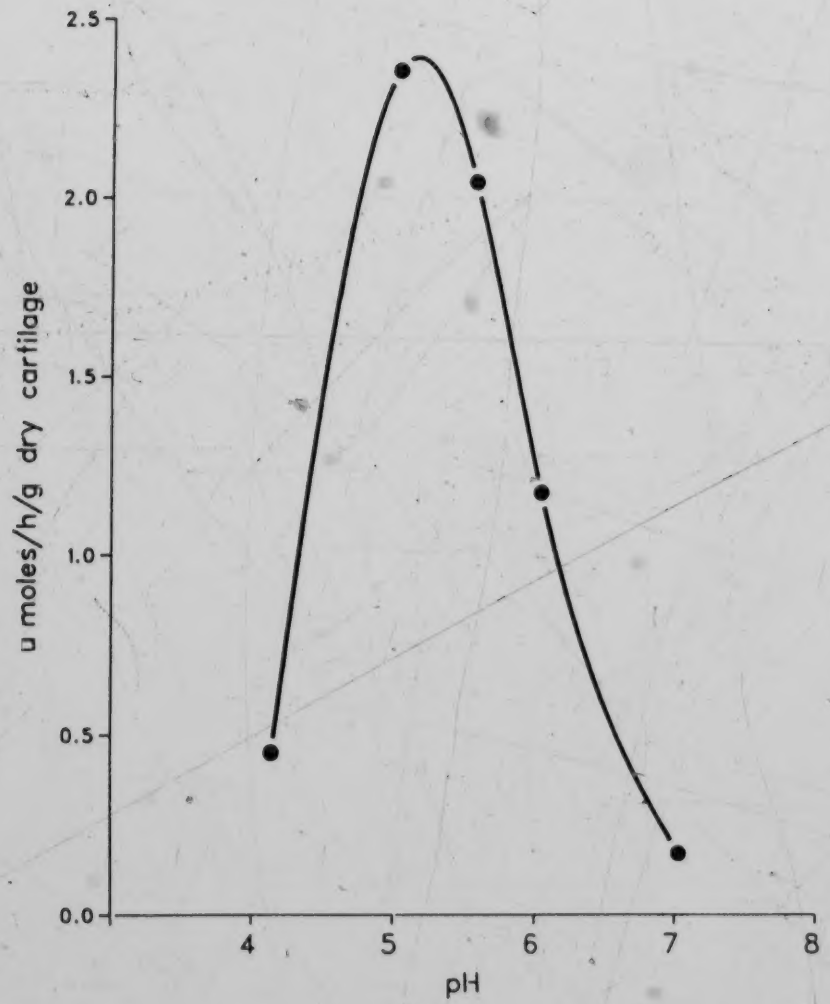


Figure V.2 Effect of pH on the hydrolysis of Z-Arg-Arg-MNA by articular cartilage extract

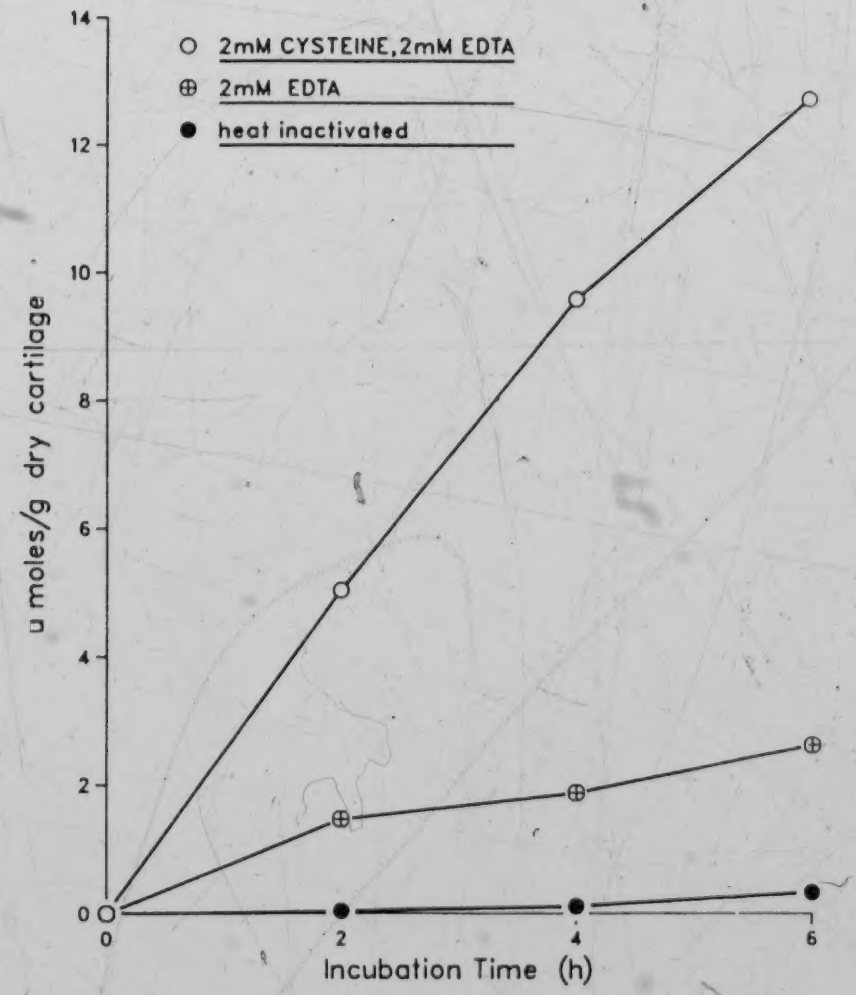


Figure V.3 Effect of cysteine and heat treatment on the hydrolysis ofZ-Arg-Arg-MNA by articular cartilage extract

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VI. EFFECTS OF GLYCOSAMINOGLYCANPOLYSULFATE TREATMENT ON SOUNDNESS, HYALURONIC ACID CONTENT OF SYNOVIAL FLUID AND PROTEOGLYCAN AGGREGATE SIZE IN ARTICULAR CARTILAGE OF LAME BOARS

A. INTRODUCTION

Because leg weakness is of complex etiology, very little progress has been made towards its prevention or cure. Fredeen and Sather (1978) reported that housing of boars in large dirt lots resulted in some recovery from lameness. However a study by Nakano et al. (1981) demonstrated no significant improvement in gross cartilage soundness of lame boars housed under similar conditions, although some evidence of cartilage repair was observed. In the study by Fredeen and Sather (1978), increased voluntary exercise or a change from concrete to dirt flooring may have caused the apparent improvement in walking condition. Enforced exercise reduced the incidence of some fore-leg abnormalities in swine (Perrin and Bowland 1977) but there is little information available concerning the extent of voluntary exercise of swine under various housing conditions and the effect of such activity on the locomotory system.

Since very few management practises can be employed to prevent leg weakness, there is still a need for methods to control the problem. Corticosteroids have traditionally been used in the treatment of non-infectious lameness in swine but their long term use is detrimental to joint cartilage integrity. Repeated intra-articular injections of hyaluronic acid have given variable results in the treatment of equine degenerative joint disease (Nizolek and White 1981) and recovery rates of over 50% have been reported. The beneficial response to hyaluronic acid administration might suggest a deficiency of this glycosaminoglycan in the synovial fluid of lame animals and a resultant failure of soft tissue lubrication. A major disadvantage of intra-articular injection is the direct invasion of the joint space and the potential for subsequent joint infection. An alternative approach is the stimulation of endogenous synthesis of hyaluronic acid by the synoviocytes. Glycosaminoglycanpolysulfate (GAGPS) stimulates the synthesis of hyaluronic acid by cultured human synoviocytes (Verbuggen and Veys 1976)

and intramuscular administration of GAGPS to humans suffering from rheumatoid arthritis increased the concentration of hyaluronic acid in the synovial fluid (Verbuggen and Veys 1979). In vitro studies indicate that GAGPS inhibits the activity of some lysosomal enzymes including three keratin sulfate-degrading enzymes (Greiling and Kaneko 1973) and cathepsin B (Kruze et al. 1976). It also stimulates proteoglycan synthesis (Kleesiek 1981 as cited by Muller et al. 1983). Nakano et al. (1985) reported that proteoglycans from severely osteochondrotic joints were less aggregated and contained a greater proportion of small monomers than those from normal joints. The enzymes responsible for such proteoglycan degradation have not been identified but normal articular cartilage of growing swine contains significant cathepsin B-like activity (Brennan et al. 1985) which can degrade the core protein of articular cartilage proteoglycan aggregates (Morrison et al. 1973). Therefore, the inhibitory effect of GAGPS on proteoglycan-degrading enzymes might cause a change in the structure of articular cartilage proteoglycans.

The objective of this experiment was to investigate the effect of repeated intra-muscular administration of GAGPS on articular cartilage and leg soundness scores, proteoglycan aggregate size and synovial fluid hyaluronic acid concentration in lame boars.

B. MATERIALS AND METHODS

Eighteen boars (101 kg bodyweight) which had been culled because of locomotory problems were obtained from Pig Improvement Company, Acme, Alberta. Three days after delivery to the University of Alberta swine unit, leg soundness of both hind and fore legs of the boars was scored on a scale from one (normal) to six (paralysis of hind legs; lameness of fore legs). The 18 boars were equally assigned to two treatments based on initial weight and leg soundness score. The animals were penned in individual 2.4 x 1.2 m pens with woven wire flooring. A 16% crude protein diet was allowed ad libitum. Water was available at all times through nipple drinkers in each pen. One group of nine boars was injected intramuscularly with an aqueous solution of GAGPS (Luitpold Werk, 8000 Munich, Federal Republic of West Germany) on days 0, 5, 10, 15, 20, and 25. A dose of 5.2 mg/kg bodyweight$^{0.75}$ was selected

because a similar level was used successfully in the treatment of human (Muller et al. 1983) and equine (Dr. L. Goldman, Luitpold werk, pers. comm.) degenerative joint disease. An equivalent volume of sterile saline solution was also administered intramuscularly to each of nine control boars at five day intervals. After a 27 day test period the boars were slaughtered by mechanical stunning and exsanguination. Leg bones were immediately removed with elbow and knee joints intact and maintained at 4°C during synovial fluid sampling. Synovial fluid samples were collected from the elbow and knee joints (Brennan et al. 1985) and analysed for hyaluronic acid content (Jourdain et al. 1979). Limb bones were frozen at -20°C.

The articular cartilage of the distal femur, distal humerus, proximal ulna and proximal radius and the distal ulnar epiphysis were each scored on a scale from 1 (apparently normal) to 6 (cartilage partially or completely separated from subchondral bone). Approximately 500 mg of cartilage was removed from the cranial aspect of the medial femoral condyles of each of 4 control and 4 treated boars. Each of the sites which were sampled were moderately osteochondrotic (score 3) with some evidence of tissue softening. These samples were diced (less than 2 mm^2) using a scalpel and proteoglycans were extracted with 5 mL of solution containing 4.0 M guanidinium chloride, 0.05 M sodium acetate (Heinegard 1972), 0.005 M benzamidine hydrochloride, 0.1 M 6-aminohexanoic acid (Oegema et al. 1975) and 0.01 M disodium EDTA adjusted to pH 5.8. Samples were extracted for 60 h on a tube rotor at 4 °C. Tubes were then centrifuged and the supernatant was filtered (glass wool) into dialysis bags. The residual cartilage was resuspended in 3 mL of extracting solution, centrifuged and the supernatant collected. The combined supernatants were dialysed against 10 volumes of 0.005 M sodium acetate buffer (pH 5.8) for 24 h. The dialysates were combined with washings from the dialysis bags and adjusted to a density of 1.5 g/mL using CsCl. The solutions were ultracentrifuged at 95,000 x g (37K) for 48 h at 10 °C using a Beckman L2-65B ultracentrifuge with a 60 Ti angle rotor. The upper three-fifths (A2) and the lower two-fifths (A1) of each centrifugate were separated using a Pasteur pipette. The densities of fractions A1 and A2 were 1.60 g/mL and 1.51 g/mL respectively. Both fractions were dialysed against five changes of distilled water for 60 h at 4°C and freeze-dried.

The cartilage residue remaining after the guanidinium chloride extract was dried in acetone (Brandt and Muir 1969) and digested with papain (5 mg of twice crystalized enzyme per g dry cartilage) for 48 h at 60°C (McDevitt and Muir 1976). The digests were centrifuged and the supernatants were diluted to 50 mL with 0.15 M sodium acetate buffer (pH 6.8). Uronic acid content of 0.5 mL aliquots of the diluted digest was determined by the carbazole reaction (Bitter and Muir 1962).

Gel chromatography of fraction A1 proteoglycans was performed as described by Nakano et al. (1985). A Sepharose 2B gel (Pharmacia, Upsaala, Sweden) was packed in a glass column (0.8 cm internal diameter) and the final packed column height was 133 cm. Proteoglycan aggregate from normal swine articular cartilage and glucuronolactone were used to determine void volume (V0) and total volume (Vt) of the column respectively. Fraction A1 proteoglycans were dissolved in the eluting buffer and applied to the column. The column was eluted with 0.5 M sodium acetate pH 5.8 at a flow rate of 4 mL/h. Fractions (0.75 mL) were collected using a fraction collector and analysed for uronic acid content by the carbazole reaction (Bitter and Muir 1962).

The percentage of uronic acid excluded from the column, appearing in the first peak (aggregated proteoglycans) or retarded in the column were calculated. The K_{ave} of the retarded peak was calculated as (Vt-Ve)/(Vt-V0), where Ve was the eluant volume.

Data were analysed by a oneway analysis of covariance with initial leg soundness score included in the statistical model as a covariate (Steel and Torrie 1980).

C. RESULTS

Leg soundness score was significantly ($P<0.05$) improved and there was a tendancy ($P<0.06$) towards an increased hyaluronic concentration in the synovial fluid of the elbow joints of GAGPS-treated boars (Table VI.1). There was no significant effect of GAGPS administration on average daily gain, average daily feed intake or gain/feed of boars during the 27 day test period. Initial leg soundness score had a significant effect on final leg soundness score ($P<0.01$), hyaluronic acid content of the knee joint synovial fluid ($P<0.05$)

and average daily gain ($P<0.05$) of boars and was therefore removed by covariate analysis. There was no significant ($P>0.10$) effect of GAGPS therapy on cartilage soundness scores for the femoral or humeral condyles, the proximal radius or the distal ulnar epiphysis (Table VI.2).

Of the 63% of cartilage uronic acid which was extracted with guanidinium chloride, approximately 90% was recovered in fraction A1. Less than 10% of uronic acid was excluded from the column (fractions 20 to 26) and less than 20% of the uronic acid was eluted in the form of aggregated proteoglycans (fractions 20 to 31) (Table VI.3). The K_{ave} of the retarded peak was 0.34 and 0.32 for the control and GAGPS-treated boars respectively ($P>0.05$). GAGPS treatment caused a significant increase ($P<0.05$) in the percentage of total fraction A1 proteoglycans which was aggregated (Figs 1 and 2).

D. DISCUSSION

The results of this study indicate that GAGPS therapy can improve the walking condition of lame boars. Many studies indicate that a similar response occurs in humans suffering from degenerative joint disease (Fujimoto and Ueno 1973; Rau et al. 1977; Siegmeth and Radi 1983). The mechanisms responsible for the observed improvement in walking condition in this and other studies are not clear. Verbruggen and Veys (1979) reported that in humans treated with GAGPS (125 mg intramuscularly), hyaluronic acid concentrations in the synovial fluid of the knee joint were significantly increased two and four days post-injection but returned to basal levels after six days. In the present study, boars which were slaughtered 48 h after the last GAGPS injection had an elevated ($P<0.06$) hyaluronic acid concentration in the synovial fluid of the elbow joint. Severe lameness could be associated with a lack of synovial fluid hyaluronic acid as indicated by a significant ($P<0.05$) partial correlation ($r=-0.49$) between initial soundness score for the hind legs and hyaluronic acid concentration in the synovial fluid of the knee joint. Hyaluronic acid which accounts for most of the viscosity of synovial fluid, is responsible for the efficient lubrication of tendons, ligaments, menisci and the synovial membrane but it is not essential for the normal lubrication of the

articular cartilage surface (Swann et al. 1974). Poor lubriction of the peri-articular tissues may cause considerable stiffness (Radin et al. 1971 as cited by Swann et al. 1971) and improved soft tissue lubrication may cause an appreciable improvement in gait. A recent review of the treatment of equine degenerative joint disease indicates that hyaluronic acid therapy is quite effective (Nizolek and White 1981). Treatment of surgically induced fractures of the radial and carpal bones of horses by administration of hyaluronic acid to the intercarpal joint increased the weight-bearing capacity of the affected limb (Auer et al. 1980). It has been reported that horses recovered from osteoarthritis after intra-articular hyaluronic acid administration (Rose 1979; Asheim and Lindblad 1976). GAGPS therapy may be the best method of increasing the hyaluronic acid concentration in the synovial fluid because the risk of joint infection is reduced. In addition, the stimulation of endogenous synthesis of hyaluronic acid may be preferable to the administration of exogenous hyaluronic acid which may differ in molecular weight or viscosity.

A reduction in proteoglycan aggregate size occurs in osteochondrotic swine articular cartilage (Nakano et al. 1985). It has been suggested that GAGPS inhibits cathepsin B activity (Kruze at al. 1976) and stimulates proteoglycan synthesis (Kleesiek 1981 as cited by Muller et al. 1983) in articular cartilage. Therefore, proteoglycan aggregate size was used in this study as an indicator of the net effect of GAGPS therapy on the proteoglycans of osteochondrotic articular cartilage. The results of proteoglycan analysis indicate a high percentage of non-aggregated proteoglycan in osteochondrotic articular cartilage from both control and treated boars. Nakano et al. (1985) reported that in the case of proteoglycan from osteochondrotic joints, as little as 23% of total fraction A1 uronic acid was excluded from a Sepharose 2B gel. In the present experiment, an average of only 7.8% of fraction A1 uronic acid was excluded from the gel column. Simunek and Muir (1972) analysed proteoglycan from lame and normal 25-week old pigs and concluded that lameness caused a significant reduction in the proportion of uronic acid which was excluded from a Sepharose 2B gel column. Thus, the severity of lameness may have influenced the chromatographic properties of articular cartilage proteoglycan in this experiment. The compressive strength of joint cartilage is largely

determined by its proteoglycan content and structure (Muir 1977). Therefore, the improvement in proteoglycan aggregation observed in the joint cartilage of the GAGPS-treated boars might have improved the weight-bearing capacity of the joint. However, the severity of osteochondrosis at each of the joint sites examined was not significantly different among treatments.

In conclusion, GAGPS administration significantly improved the leg soundness score of lame boars possibly due to an elevation of hyaluronic acid concentration in the synovial fluid. An improvement in the structure of articular cartilage proteoglycan also occurred in response to GAGPS treatment but this did not result in a significant improvement in joint soundness score.

Table VI.1 Effect of GAGPS treatment on leg soundness scores, performance and synovial fluid hyaluronic acid concentration of lame boars

	Treatment			
	Saline	GAGPS	SE	SIG
Initial leg soundness score:†				
Front legs	2.22	2.56	0.48	NS
Hind legs	4.00	3.78	0.56	NS
Overall	3.11	3.17	0.41	NS
Final leg soundness score:				
Front legs	3.11	2.00	0.46	P<0.06
Hind legs	4.44	3.33	0.34	P<0.06
Overall	3.78	2.67	0.31	P<0.05
Synovial fluid hyaluronic acid (mg/ mL):				
Elbow joint	1.78	2.25	0.15	P<0.06
Knee joint	1.51	1.71	0.18	NS
Initial weight, kg	101.4	101.5	2.1	NS
Final weight, kg	121.3	123.1	3.35	NS
Average daily gain, kg	0.73	0.80	0.13	NS
Average daily feed, kg	2.33	2.77	0.25	NS
Gain/feed, kg	0.27	0.28	0.02	NS

†Scored on a scale from 1 (normal) to 6 (paralysis of hind limbs; lameness of fore limbs)
SE, standard error of mean
SIG, significance of difference between means (NS, P>0.10)

Table VI.2 Effect of GAGPS treatment on cartilage soundness scores† of boars

Site	Treatment		SE	SIG
	Saline	GAGPS		
Medial femoral condyle	2.33	2.75	0.30	NS
Lateral femoral condyle	1.67	1.19	0.24	NS
Medial humeral condyle	1.72	1.94	0.40	NS
Lateral humeral condyle	2.00	1.75	0.48	NS
Proximal Radius	1.44	1.31	0.26	NS
Distal ulnar epiphysis	1.83	2.25	0.31	NS

SIG, significance of difference between means (NS, $P>0.10$)
SE, standard error of mean
†Scored from 1 (sound) to 6 (severely osteochondrotic)

Table VI.3 Effect of GAGPS therapy on the chromatographic properties (Sepharose 2B gel) of fraction A1 proteoglycans from osteochondrotic swine articular cartilage

	Treatment			
	Saline	GAGPS	SE	SIG
Excluded, %	6.00	9.60	1.22	NS
Aggregated, %	10.7	19.5	1.52	*
K_{ave} of retarded peak	0.34	0.32	0.03	NS

SE, standard error of mean
SIG, significance of difference between means (NS, $P>0.05$)
* $P<0.05$

Figure VI.1 Sepharose 2B column chromatogram of fraction A1 proteoglycans from ostoechondrotic articular cartilage of a lame boar which was treated with saline. Aggregated proteoglycan and glucuronolactone were used to determine void volume (Vo) and total volume (Vt) of the column, respectively.

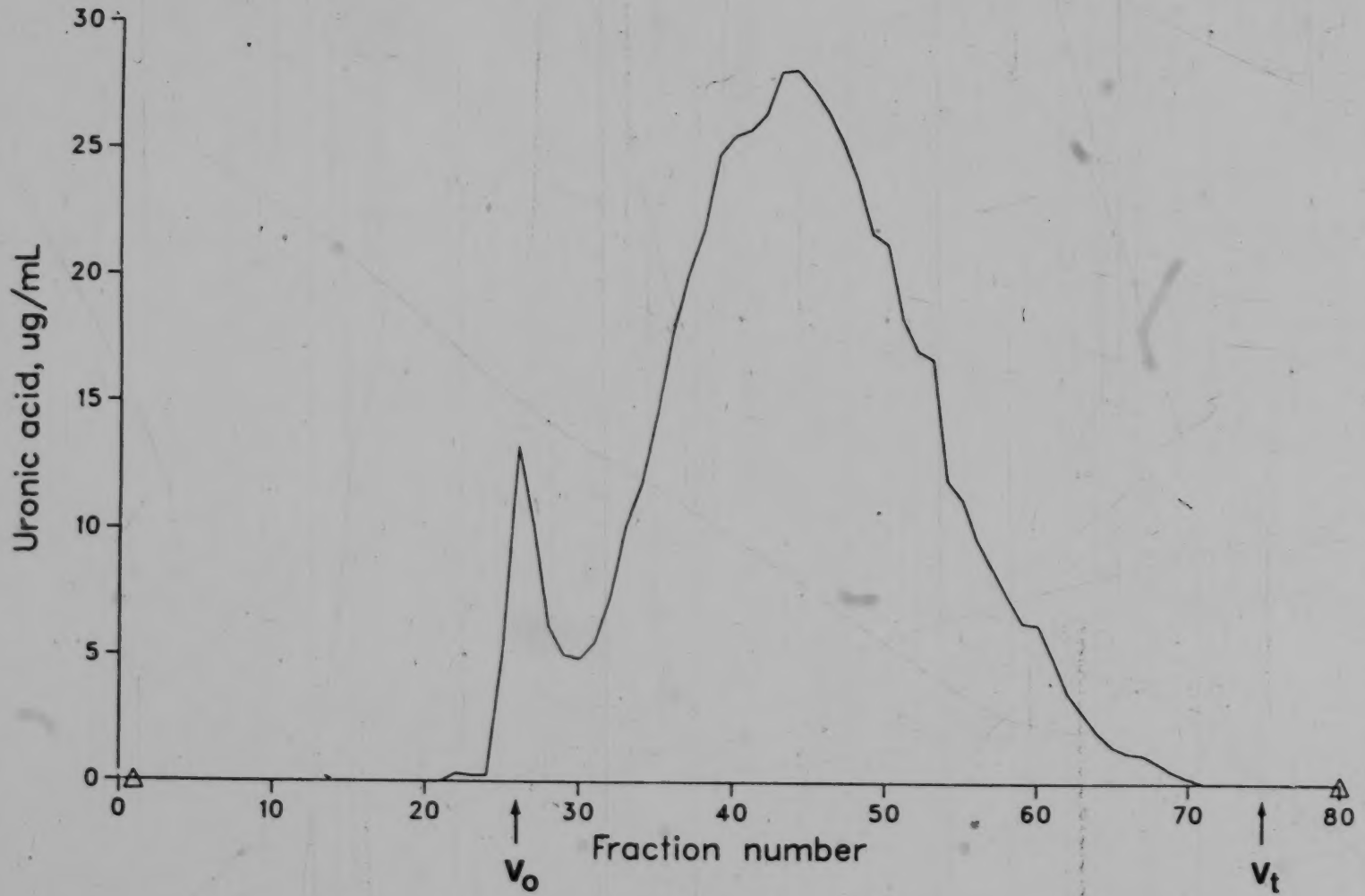
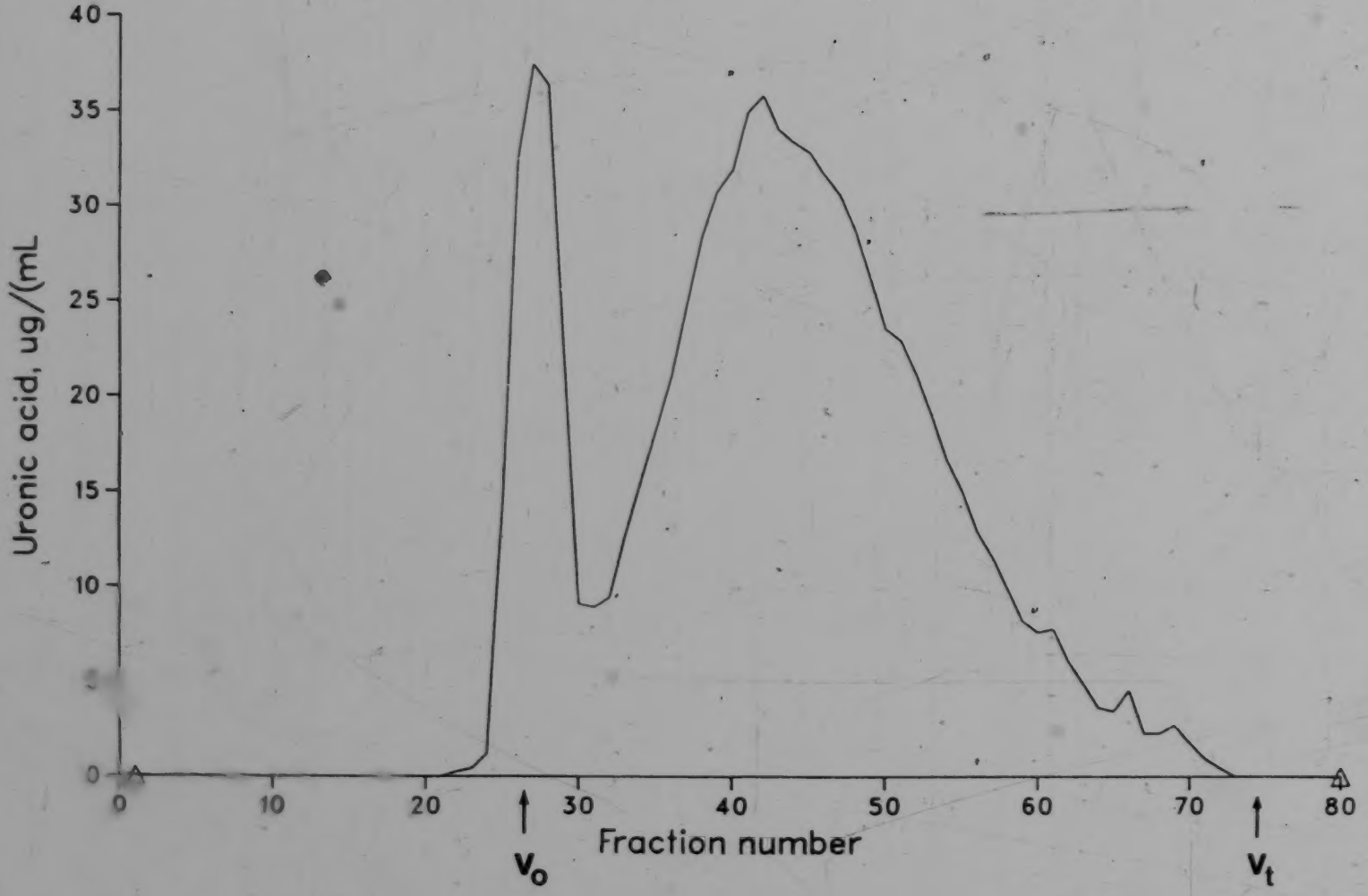

Uronic acid, ug/mL
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
Fraction number
V_o
V_t

Figure VI.2 Sepharose 2B column chromatogram of fraction A1 proteoglycans from ostoechondrotic articular cartilage of a lame boar which was treated with glycosaminoglycan polysulfate; Vo, void volume ; Vt, total volume.


Uronic acid, ug/(mL
40
35
30
25
20
15
10
0
0
10
20
30
40
50
60
70
80
Fraction number
V_o
V_t

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VII. GENERAL DISCUSSION

The first experiment reported herein indicated that the severity of osteochondrosis was not significantly reduced by the provision of elevated dietary calcium and phosphorus (Ca-P) levels. Large increases in bone bending moment were achieved by feeding Ca-P levels in excess of National Research Council (NRC 1979) recommendations but there was no significant correlation between joint lesion severity and bone bending moment. It is therefore suggested that bone bending moment is of questionable value as a criterion for assessing the structural soundness or adequacy of skeletal development in swine. Average daily gain, average daily feed intake and feed/gain of pigs were not significantly affected by dietary Ca-P levels. These results are in agreement with previous studies which indicate that osteochondrosis does not result from a reduction in bone mineralisation (Nakano et al. 1981) and that the disease cannot be prevented by feeding elevated dietary Ca-P levels (Grondalen 1974). It has been suggested by Olsson (1978) that osteochondrosis results primarily from the abnormal growth and maturation of chondrocytes and should therefore be termed dyschondroplasia. Further evidence that osteochondrotic lesions may be primarily cartilagenous has been presented by Nakano et al. (1986). Cartilagenous lesions may subsequently cause a failure of vascular penetration, If this hypothesis is true, then the lesions observed in bone must be secondary to those in cartilage and therefore not preventable or reversable by increasing Ca-P supply to subchondral bone. Interruptions of metaphyseal blood supply due to mechanical stress have also been cited as an apparent primary cause of osteochondrotic lesions of the distal ulnar epiphysis (Grondalen and Grondalen 1974). When osteochondrotic lesions result primarily from a failure of vascular penetration, an elevated supply of Ca and P to the invading vascular loops at the chondro-osseous junction might be beneficial if these minerals stimulated vascular penetration itself. The data presented in chapter II indicate however that this did not occur.

Many investigations of the role of nutritional factors in the etiology of osteochondrosis are based on the hypothesis that if a deficiency of a mineral or vitamin is associated with abnormalities of bone, joints or the general locomotary system, then the

provision of elevated levels of these nutrients (in excess of NRC (1979) recommendations) may prevent the development of lameness. There is a considerable volume of evidence indicating that this hypothesis is not true; examples include Ca and P (Grondalen 1974), manganese, copper (Walker et al. 1966) and vitamins A, D, E (Grondalen 1974; Reiland 1978) and C (Walker et al. 1966; Nakano et al. 1983). However, a recent report indicating that osteochondrotic lesions are present in swine at birth suggests the possibility that maternal nutrition is a potential factor in the etiology of osteochondrosis (Hill et al. 1985).

The severity of elbow joint lesions was much greater in males than in females (Chapter II), a phenomenon which has been reported in several other species. Because the medial humeral condyle seems to be predisposed to osteochondrosis dissecans, a condition which is partly traumatic in origin, mechanical factors might have contributed to the greater severity of osteochondrosis of the elbow joint in boars. The data presented in chapters II and III indicated that average daily bodyweight gain and lean tissue growth rate were not significantly associated with the severity of osteochondrosis. Therefore, the observed sex effects were not attributable to growth rate differences among the sexes.

There is considerable evidence that a predisposition to mechanical stress is an important factor in the etiology of osteochondrosis. The predominance of lesions on the weight-bearing sites of the medial condyles of the femur and humerus and the positive correlation between lesion severity in the articular cartilage of the proximal radius and in the medial humeral condyle (Chapter II) suggest the involvement of mechanical factors. Kincaid et al. (1985) suggested that cartilage canals in the humeral condylar cartilage of growing pigs may be associated with sites of mechanical weakness. The distribution of osteochondrotic lesions is random at birth (Hill et al. 1985) but at 6 months of age, the weight-bearing sites of the elbow and knee joints have the greatest incidence and severity of lesions (Nakano et al. 1979). Therefore some mechanical factors must influence endochondral ossification at these sites during the first 6 months of life.

It is well established that moderate increases in weight-bearing by the front legs of swine results in a severe disruption of ossification of the distal ulnar growth plate (Walker et

al. 1966; Grondalen and Grondalen 1974). The lesions produced are similar to osteochondrotic lesions. The objective of the experiment described in chapter V was to determine if similar changes occurred in the articular cartilage of the humeral condyles. Mechanical stress caused an erosion of the articular cartilage surface of the medial humeral condyle which was associated with a localized loss of proteoglycan staining. Cathepsin B-like activity and uronic acid content of humeral condylar cartilage were similar among control and mechanically stressed animals. There was no effect of loading on the hyaluronic acid concentration in the synovial fluid of the elbow joint, indicating that the integrity of the synovial membrane was not affected by the joint stress imposed. It was concluded that the adverse effect of mechanical stress on the articular cartilage was of a focal nature with no extensive loss of uronic acid or increase in cathepsin B-like activity. Because several studies indicate that a variety of cartilaginous lesions can cause a failure of endochondral ossification, it is likely that focal degeneration of articular cartilage induced by mechanical stress could be manifested as osteochondrotic lesions as bone growth progresses. It is therefore recommended that greater care be taken in the handling and transport of swine due to the fragility of immature joint cartilage.

It has been difficult to identify specific housing, management and anatomical factors which might support the mechanical stress hypothesis. Muscle distribution within the proximal pelvic group and muscle to bone ratio were poor predictors of the severity of osteochondrosis (Chapter III). Modern confinement housing facilities for swine include a diversity of floor types designed primarily for convenience of husbandry rather than for animal needs. A woven wire floor caused more severe foot lesions in starter pigs (22 kg bodyweight) than did a plastic coated expanded metal floor (Chapter IV). Floor type did not, however, have any significant effect on joint soundness and there was no significant association between foot lesions and joint lesions. Perrin et al. (1978) also observed no significant effect of floor type on the severity of osteochondrosis in swine.

In vitro studies indicate that the metabolic activity of the chondrocytes is very responsive to the frequency and duration of cartilage compression (Chapter V) but the

mechanism by which the cell responds to changes in pressure is not known. The response of the chondrocyte to pressure changes suggests that behaviour of swine (time spent lying, exercising) could influence the synthesis and/or degradation of matrix proteoglycans. Therefore, animal behaviour may interact with management practises and housing conditions to effect the severity of osteochondrosis.

Mechanical stress may be involved in many stages of the development of osteochondrosis. Trauma or static stress are potential causes of cartilage fracture and chondrocyte damage but they may also be primary causes of failure of vascular penetration (Grondalen and Grondalen 1974) or of osteochondrosis dissecans. The increased thickness of osteochondrotic articular cartilage and its reduced proteoglycan aggregate size (Nakano et al. 1985) could increase the susceptibility of the joint to mechanical stress. One of the earliest degenerative changes observed in the joint cartilage of young pigs is the focal loss of proteoglycans which are necessary for the maintenance of the compressive strength of the tissue.

Because the culling of lame boars represents a considerable economic loss to swine breeders, there is a need for measures to prevent or control the problem. The data presented in chapter VI indicate that glycosaminoglycanpolysulfate (GAGPS) treatment significantly improved structural soundness, hyaluronic acid concentration in the synovial fluid of the elbow joint and the structure of articular cartilage proteoglycans in lame boars. Reduced concentrations of hyaluronic acid in the synovial fluid of the knee joints were associated with an increased severity of lameness of the hind limbs. Since hyaluronic acid is responsible for the efficient lubrication of the soft tissues of the joint, its concentration in synovial fluid may have a large influence on leg soundness scores.

Proteoglycan aggregates bind a large volume of water and give cartilage its resilience and weight-bearing capacity. The improvement in proteoglycan aggregate size in response to GAGPS therapy might increase the compressive strength of articular cartilage. It is not known however, if the improvement in proteoglycan structure is of sufficient magnitude to influence the mechanical properties of the articular cartilage.

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